Exhibit Q

IN THE UNITED STATES DISTRICT COURT
FOR THE EASTERN DISTRICT OF NEW JERSEY

IN RE JOHNSON & JOHNSON TALCUM POWDER PRODUCTS MARKETING, SALES PRACTICES, AND PRODUCTS LIABILITY LITIGATION	MDL NO. 16-2738 (FLW) (LHG)
THIS DOCUMENT RELATES TO ALL CASES	

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Thursday, April 11, 2019

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Videotaped Deposition of KELLY TUTTLE, Ph.D., held at the Hilton Fort Worth, 815 Main Street, Fort Worth, Texas, commencing at 9:08 a.m., on the above date, before Michael E. Miller, Certified Court Reporter, Registered Diplomate Reporter, Certified Realtime Reporter and Notary Public.

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DEPOSITION EXHIBITS

DEPOSITION EXHIBITS

PROCEEDINGS

(April 11, 2019 at 9:08 a.m.)

THE VIDEOGRAPHER: We're now on the record. My name is David Lane, videographer for Golkow Litigation Services. Today's date is April 11th, 2019. The time is 9:08 a.m.

This deposition is taking place in Fort Worth, Texas, in the matter of Talcum Powder Litigation MDL. Our deponent today is Kelly Tuttle Ph.D. Our counsel will be noted on the stenographic record.

The court reporter today is Mike Miller, who will now swear in the witness.

KELLY TUTTLE, Ph.D.,
having been duly sworn,
testified as follows:

BY MR. SOILEAU:

Q. Good morning, Dr. Tuttle.

A. Good morning.

Q. I am Rudie Soileau. We met just a few moments ago. I'm an attorney from

Lake Charles, Louisiana. I'm appearing today on behalf of women and the families of women who maintain that the use of Johnson & Johnson talcum powder products led to the development of ovarian cancer.

(Whereupon, Deposition Exhibit Tuttle-1, Tuttle Expert Report, was marked for identification.)

BY MR. SOILEAU:

Q. Let me begin by handing you an exhibit that I have marked as Tuttle Exhibit 1 and ask you to identify that for me.

A. This is the report that I drafted regarding this litigation.

Q. Very good. You understand that you are under oath today?

A. I do.

Q. And you have been deposed previously?

A. Yes, I have.

Q. Dr. Tuttle, I intend for all of my questions today to be clear and fair. If a question is for any reason not clear to you

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or if it does not seem fair, tell me. And if you tell me that, I'll do whatever is necessary to make sure that my questions seem clear and fair, okay?

A. Sounds good.

Q. If you do not say anything, I'm going to assume that my questions are clear and you are answering the question I've asked. Fair enough?

A. Yes.

MR. FROST: Rudie, I just have one question on the report. I just want to make sure it's purposeful. I note that the appendices are not in the back of the one that's marked as Exhibit 1. I just want to make sure that's purposeful.

MR. SOILEAU: Yes.

MR. FROST: Okay.

MR. SOILEAU: I included the entire -- and let me ask the doctor.

BY MR. SOILEAU:

Q. Exhibit 1 is the entirety of the report itself as well as your references,

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but none of the appendices, correct?

MR. FROST: Didn't mean to interrupt your flow. I just wanted to make sure that that was on purpose.

MR. SOILEAU: That's fine.

A. Cursory glance through, yes, this appears to be my complete report, with the exception of the appendices.

BY MR. SOILEAU:

Q. Very good.

Were you asked, Doctor, by the attorneys for Johnson & Johnson to review available information and offer an opinion addressing any relationship between Johnson & Johnson talcum powder products and ovarian cancer?

A. I was asked by attorneys to look at the scientific literature and the body of science to determine whether the body of science supports a causal association between perineal talcum powder exposure and ovarian cancer.

Q. Very good.

Let me show you one of the

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appendices that counsel referred to a moment ago.

(Whereupon, Deposition Exhibit Tuttle-2, Tuttle Previous Four Years Testimony, was marked for identification.)

BY MR. SOILEAU:

Q. I've marked this document as Tuttle Exhibit 2. Do you recognize that?

A. Yes, I do.

Q. Do each of the instances listed in Appendix E, which is now Tuttle Exhibit 2, arise from a release, a spill or some other unintended event?

A. No, they do not.

Q. Which one does not?

A. Let's see. There are several on here that are related to a spill or release or emergency response, and then there are others that are toxic tort litigation.

Q. Which of the captions that are listed on Tuttle Exhibit 2 involve toxic tort litigation?

A. So I'll just start at the

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beginning. The first one, Baukholt v. A.O. Smith. The second one, Wilson v. Eighty-Eight Oil. The third, Readwin v. Aurora Pumps. Then going to the fifth, Hudson v. Covestro. Then going to the second page, the second one, Messel v. Aurora Pump; the third, Howe v. Aurora Pump; and the fourth, Bradd v. Aurora Pump.

Q. Do the four matters that reference Aurora Pump all involve the same issue generally from the perspective of a toxicologist?

A. Yes, generally.

Q. And what was the substance or agent involved in the four Aurora Pump cases?

A. Those were involved in asbestos litigation.

Q. Were the plaintiffs in the Aurora Pump litigations that are listed on Tuttle Exhibit 2 workers who had been occupationally exposed to asbestos?

A. I would have to go back and look at the individual cases. I don't recall.

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Q. Okay. The Baukholt matter, the first one listed on Exhibit 2, what did that litigation involve?
A. That also was asbestos litigation.
Q. And what about Wilson v. 88 Oil?
A. That involved a number of potential industrial hygiene and toxicological exposures resulting from an oil transloading facility.
Q. Hudson, which is also on the first page of Tuttle Exhibit 2, what was the agent or substance at issue in the Hudson matter, Doctor?
A. Hudson v. Covestro involved spray foam insulation.
Q. Was there any allegation that the spray foam included as a constituent asbestos fibers?
A. No.
Q. Okay. Have you ever been asked to review available information and offer an expert opinion involving a manufactured

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retail product other than this case?
A. In regards to litigation, not that I recall specifically. I guess the spray foam insulation would be considered a manufactured product.
Q. But not one sold retail, correct?
A. Not that I can recall, no.
Q. The product that we're talking about now, talcum powder products, you understand those were manufactured by Johnson & Johnson?
MR. FROST: Objection.
BY MR. SOILEAU:
Q. Is that your understanding?
A. My understanding is that talcum powder is -- or talc specifically is naturally occurring and is sold in any number of options. In this particular case my research is around perineal talcum powder and it is my understanding that Johnson & Johnson sells talcum powder products.
Q. From your perspective and your work in this case, would it be appropriate to

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consider Johnson & Johnson the manufacturer of the talcum powder products that we are discussing today?
MR. FROST: Objection.
A. So I was asked to review the body of science and the scientific literature, and so in regards to the scientific literature, a lot of the studies and things are -- do not specify what form of talcum powder is involved in their studies or in their assessments, so this is an assessment of the body of science as a whole.
BY MR. SOILEAU:
Q. I see.
So from your perspective, has the work that you've done in this case focused on the body of science and not the actual products that Johnson & Johnson sells to citizen consumers?
MR. FROST: Objection.
A. Well, as I said, I assessed the body of science, and a large number of studies do not specify the type of talcum powder. That being said, there are examples

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such as the FDA and others where Johnson & Johnson products specifically were tested or examined.
But in regards to epidemiologically or otherwise, it revolves around talcum powder products as a whole and the scientific literature as a whole.
BY MR. SOILEAU:
Q. Do you agree that the essence of toxicology is the study of adverse effects of agents on organisms?
A. Toxicology is the study of the dose and response of various products, either man-made or natural, and their potential adverse effects on the environment, on the human body, on animals.
(Whereupon, Deposition Exhibit Tuttle-3, Chapter from Reference Guide on Toxicology, was marked for identification.)
BY MR. SOILEAU:
Q. Let me show you an additional exhibit. I've marked this as Exhibit 3. This is a chapter from the reference manual

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that is noted in your report and included in your reference materials.
Do you recognize this chapter?
A. Yes.
Q. Do you have a copy of the reference manual somewhere at home or in your office?
A. It's in our corporate office in Little Rock, Arkansas, yes.
Q. Where do you office?
A. I office out of my home here outside of Fort Worth.
Q. I see.
Turn to page 636, if you would. I'm going to put this on the ELMO as well, but I'm focusing on the second full paragraph on page 636, and the second sentence which says: It -- referring to toxicology -- it is the study of the adverse effects of chemical and physical agents on living organisms.
Did I read that correctly?
A. Yes, you read that correctly.
Q. Do you agree with that statement?

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A. As I said, toxicology is the study of compounds and their potential for adverse health effects on living organisms as well as the environment.
Q. Is that a yes to my question?
A. Well, again, I said that toxicology is the study of the potential adverse health effects of chemicals, be them man-made or naturally occurring, on living organisms, including the environment.
Q. Yes, Doctor. I did hear your answer, but my question was: Do you agree with the summary statement that I read from the reference manual, Tuttle Exhibit 3?
MR. FROST: Objection.
A. Again, as I said, in addition, I said that toxicology is the study of adverse effects of chemical and physical agents, be them natural or man-made, on living organisms, that includes animals and the environment.
BY MR. SOILEAU:
Q. Let me show you another exhibit then.

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(Whereupon, Deposition Exhibit Tuttle-4, Excerpt from The Basic Science of Poisons, was marked for identification.)
BY MR. SOILEAU:
Q. I'll mark this as Exhibit 4. This is a one-page excerpt from a textbook. The cover of the textbook is on the first page.
You recognize that textbook?
A. Yes, I do.
Q. This is the ninth edition, I believe, but you cite the eighth edition as a textbook in your reference manual, correct?
A. Yes. The ninth edition is brand-new. I actually just received it the other day.
Q. I see.
Do you consider the Casarett & Doull textbook on toxicology to be a generally accepted and authoritative textbook for the field of toxicology?
A. I think as I state in my report, Casarett & Doull's is a commonly used

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toxicological textbook in teaching and education for toxicology.
Q. Do you consider it authoritative for your work as a toxicologist?
MR. FROST: Objection.
A. Again, it's commonly used for teaching toxicological principles. As far as being authoritative, that's very general. Casarett & Doull's covers a wide range of topics and a wide range of toxicological principles. I couldn't say specifically, without getting into some specifics in the textbook, on whether it would be authoritative or not, but it is commonly used for teaching toxicological principles.
BY MR. SOILEAU:
Q. Right. Doctor, we're not, of course, teaching today, at least that's not our primary purpose. We're here today to discuss your expert testimony.
I need to ask you: Do you consider it to be reliance material for your work as a toxicologist?

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A. I cite Casarett & Doull's in my report regarding toxicological principles. I certainly refer to it. I also refer to the science that it cites and the basis for what it includes in the textbook.

Q. Let's look at page 2 of Tuttle Exhibit 4. In the first paragraph, the third sentence, I want to put it on the ELMO and read it: Toxicology arose as a way to understand, prevent, mitigate and treat the potentially harmful consequences of many of the substances we are exposed to.

Did I read that correctly?

A. Yes, you read that correctly.

Q. Do you agree with that statement from the Casarett text?

A. I have not -- as I said earlier, this is the new edition, which I haven't -- I'm not versed in, I haven't read. I just received it.

Without going into more detail, I can't say whether I agree or disagree.

Q. So you're not able to agree or disagree with this statement? Just to be

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clear, I'm not asking you if it actually appears in the ninth edition. I'm simply asking you if this statement, which I have read to you from the ninth edition, is a statement that you can agree with.

A. Well, as I said, I -- without reading more in, and I'm not familiar with this new edition of the textbook, I haven't reviewed it, I can't agree or disagree.

Q. All right. Let me ask you about another statement. Just below the statement that I read is a statement that is in italics. And it says: Toxicology is the study of adverse effects of chemical, physical or biological agents on living organisms and the ecosystem, including the prevention and amelioration of such adverse effects.

Can you agree or disagree with that statement?

A. Well, again, that's the definition of toxicology as described in the textbook, and I believe that's an iteration of what I said earlier about toxicology being

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the study of potential adverse effects of agents, be them natural or man-made, on living organisms and the environment.

Q. Do you agree that the science of toxicology includes the prevention of adverse effects?

A. It depends. You know, I believe that classically and I think it even said in the reference manual we discussed earlier that toxicology is the study of poisons. A lot of that revolves around dose and understanding the role of dose in exposures to products.

It certainly can be involved in the prevention of adverse effects.

Q. I asked if you agree that the science of toxicology includes the prevention of adverse effects and you said in part, it depends. What does it depend on?

MR. FROST: Objection.

A. Well, as I said, you know, classically, and then the key tenet of toxicology is the dose makes the poison. The dose plays a key role in assessing whether an

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agent can be harmful or have a potential adverse effect.

So depending on the scenarios of the questions being asked or how toxicology is being applied, it can certainly be involved in the prevention of or the research to prevent the adverse effects.

BY MR. SOILEAU:

Q. Do you recognize the second statement I read, the italicized paragraph from the Casarett textbook?

MR. FROST: Objection.

A. I'm sorry, it's stated here in this new edition of the Casarett textbook.

BY MR. SOILEAU:

Q. Yes. Do you recognize it independent of the textbook?

A. Not specifically, no.

Q. Do you see in the second paragraph from that page, just above the quoted language, it says According to the Society of Toxicology?

A. Yes, I do.

Q. You recognize that entity that

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is the Society of Toxicology?
A. I do. I'm a member of the Society of Toxicology.
Q. Right. That's what I was going to ask.
Let me go ahead and show you what I will mark as Tuttle Exhibit 5.
(Whereupon, Deposition Exhibit Tuttle-5, Tuttle Curriculum Vitae, was marked for identification.)
BY MR. SOILEAU:
Q. An additional attachment to your report. Do you recognize this?
A. Yes, I do.
Q. Is this essentially your CV?
A. Yes.
Q. And it includes, as you just noted, your membership in the Society of Toxicology or SOT, the same entity that is referenced in this excerpt from Casarett, which is Tuttle Exhibit 4, correct?
A. Yes, that's correct.
Q. Separate from the case that brings us here today, Dr. Tuttle, have you

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ever applied the Bradford Hill methodology or a like methodology to the issue of a causal relationship for a manufactured retail product like talcum powder?
A. I use the Hill criteria throughout my work as a toxicologist.
Q. Understood.
A. I can't provide a specific example where I've used it for a manufactured product. I use it regularly in assessing the scientific literature in the body of science.
Q. In this instance, you have used the Hill methodology in part to answer the question that you described for me earlier, that is, the question that the J&J attorneys posed to you following your retention. Fair?
A. As I said, the attorneys asked me to assess the body of science and look at the scientific evidence regarding a causal association between talcum powder and ovarian cancer, for which the Hill criteria is one of the methodologies that I used in my report and discuss in my report.
Q. Yes, Doctor. Did you use the

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Hill methodology to answer the question that the J&J attorneys put to you, the same question you just summarized?
A. As I said, the Hill criteria is one of the methodologies that I include in my report for assessing the body of science and the scientific literature.
Q. Lawyers are sometimes funny about words. I understand that you're familiar with the methodology and you cite it in your report.
I wanted to know specifically: Did you apply, did you use, did you take the body of science that you referenced earlier and apply the Bradford Hill methodology to arrive at an answer to the question posed by the J&J attorneys?
MR. FROST: Objection.
A. Well, again as I said, the Hill criteria is one of the methodologies that I use in my report in assessing the body of science and the scientific literature to see what the scientific evidence supports.
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BY MR. SOILEAU:
Q. You say it is one of the methodologies that you use in your report. For what purpose did you use it in your report and in connection with your work in this matter? Let's approach it from that angle.
MR. FROST: Objection.
A. Again, I used it to assess the body of science and the scientific evidence regarding whether the scientific evidence supported a causal association between talcum powder exposure perineally and ovarian cancer.
BY MR. SOILEAU:
Q. So you used the Hill methodology to determine whether the scientific evidence supported a causal relationship or association between talcum powder exposure and ovarian cancer?
MR. FROST: Objection.
BY MR. SOILEAU:
Q. Is that correct?
A. As I said the Hill criteria is

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one of the methodologies I discuss and use in my report.
Q. I still don't know what you used it for. Can you help me?
A. Sure.
MR. FROST: Objection.
A. I used it to assess the body of science and the scientific evidence to determine whether the scientific -- what the scientific evidence supports regarding the potential for perineal talcum powder exposure to be causally associated with ovarian cancer.
BY MR. SOILEAU:
Q. And in this case, when we talk about talcum powder exposure, what products are we talking about?
A. Again, when we talked a little bit about this previously, in regards to perineal talcum powder exposure, we're dealing with cosmetic talc products. Brands and manufacturers in some of the scientific literature are not specified, so I can't speak to that.

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As I mentioned earlier, there is some testing specific to Johnson & Johnson, which is the product specifically involved today, but it is my -- generally speaking, I'm referring to cosmetic talcum powder products.
Q. I really want to understand whether a part of your focus and the work that you did in this project was on specific products, talcum powder products, sold by Johnson & Johnson. Was it?
MR. FROST: Objection.
A. I'm sorry, can you clarify that question for me a little, please?
BY MR. SOILEAU:
Q. Sure. I wanted to know whether in this project you had a specific focus on particular products sold by Johnson & Johnson, specific talcum powder products sold by Johnson & Johnson?
MR. FROST: Objection.
A. So in regards to the body of science, as I said, we -- I assessed the scientific evidence and the body of science

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regarding perineal talcum powder exposure. And in those studies and studies that I cite, they do not specify a manufacturer or product.
Other places in my report, there is research specific to Johnson & Johnson that is addressed and discussed in my report.
BY MR. SOILEAU:
Q. Do you know what products are at issue in this case?
A. It is my understanding that the Johnson & Johnson baby powder and Shower to Shower products are involved in this litigation.
Q. How were you first contacted to work in this litigation on behalf of Johnson & Johnson?
A. I was first contacted in November of last year, either by Mr. Frost or Ms. Jessica Miller. I can't remember exactly who.
Q. Very good.
Had you had any prior work or

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relationship with Johnson & Johnson before that contact in -- I think you said November of 2018?
A. I think I had met them via e-mail in maybe the month prior, but no.
Q. The e-mail that you're referring to during the month of October of 2018, that e-mail was related to your retention ultimately in this project?
A. Yes.
Q. So no other work that you have done for Johnson & Johnson in your professional career, regardless of whether it pertained to talcum powder products or some other issue?
A. I have not done any work for Johnson & Johnson previously.
Q. Okay. Do you know Dr. Nadia Moore?
A. I am familiar with Dr. Moore's name. I do not know her personally.
Q. Do you only know her name through this litigation?
A. I believe so.

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Q. Did you review her deposition?
A. Yes, I reviewed her deposition.
Q. What is her area of expertise?
A. She's a toxicologist.
Q. Do you know whether CTEH has provided any services to Johnson & Johnson other than the project that brings you and I together here today?
A. Not that I'm aware of.
Q. When you were first retained by the attorneys for Johnson & Johnson, what familiarity or knowledge did you have about the company Johnson & Johnson?
A. I know the name Johnson & Johnson. I use their products personally. Beyond that, I don't know that I had any knowledge. I had done some personal research previously about talcum powder in regards to what I'd seen in the news, but beyond that, nothing specific.
Q. Very good.
Nothing really that was aimed at the company Johnson & Johnson, only at talcum powder and some talcum powder

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products, fair?
A. I'm sorry, what do you mean, the...
Q. I asked you if you had any familiarity, and I think what you told me basically, and I just wanted to make sure I understood, that you really didn't have any specific familiarity with the company, although you had looked at the products and had some familiarity with particular products, talcum powder products, fair?
A. As I said, I know the name Johnson & Johnson. I use their products, and I had seen some of the news articles in previous years regarding Johnson & Johnson.
Q. Okay. Which products manufactured by Johnson & Johnson have you used, Doctor?
A. Oh. I would have to go through my bathroom and see what all products are there, but I --
Q. That's fair. Let me interrupt and narrow my question. I don't want to interrupt you today, but I realize that was a

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broad question because Johnson & Johnson manufactures lots of products, right?
A. Yes.
Q. I meant to include, but I admittedly did not, what talcum powder products manufactured by Johnson & Johnson have you used in the past?
A. I have talcum powder, the baby powder, for my son.
Q. You're the mother of a newborn?
A. Yes.
Q. And it's your first child?
A. It is.
Q. And you use Johnson & Johnson's baby powder on your newborn baby?
A. Yes, I do.
Q. And do you use the talcum powder?
A. I do.
Q. Okay. Had you previously used any Johnson & Johnson talcum powder products?
A. Again, I would have to go back and look specifically. I haven't looked at the ingredients in all the products that I

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use in my bathroom, so I'm not sure.
Q. Well, have you used Shower to Shower?
A. No, I haven't.
Q. Have you used any like talcum powder product, and I just mean a talcum powder product that's used for personal use?
A. Again, I would have to look at the ingredients of the products that I have in my bathroom. I don't know the specific ingredients for everything that's in my bathroom.
Q. When did you do the personal research that you referenced a few moments ago?
A. A few years ago when I had seen some news articles coming out, I just briefly on my own time got into the scientific literature and kind of looked at the issue that was being discussed.
Q. Were you working for CTEH at that time?
A. I believe so, yes.
Q. Were you married?

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A. I don't think so, no.
Q. Okay. And I don't -- perhaps it's apparent, but I don't think you know exactly when you did this research. Maybe I should have asked.
Do you know exactly when you began to conduct this personal research?
A. Not exactly, no.
Q. Is it fair to say that your best estimate would be sometime after you went to work with CTEH but before you were married?
A. Yes, I believe so.
Q. Are there any other events that would help us pin it down more specifically?
A. No, not specifically. It's a common practice of mine, so I don't know that I could really specify when I particularly researched it.
Q. What steps did you undertake, Doctor, when you conducted this personal research into talcum powder products?
A. It was just a very general cursory dive into the scientific literature,

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some PubMed searches, some Google Scholar searches, just looking at the body of science.
Q. You say cursory dive, but a cursory dive as a toxicologist, right?
A. Yes.
Q. So you did look into PubMed and Google Scholar, you said?
A. Yes.
Q. Okay. And did you make notes?
A. No, I didn't make any notes.
Q. Did you do anything to -- well, strike that.
How long did you continue to conduct this personal research?
A. I don't know how long I spent doing it. As I said, it was just a matter of personal interest. It's a common practice of mine to try to, you know, read the literature on a regular basis, so I don't know how long I spent.
Q. Did you conclude this personal research at some point prior to the time that you were contacted in October of -- or

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November of last year?
A. I don't know that there was necessarily a conclusion. As I said, I saw it. It triggered my interest. I looked into the scientific literature. You know, it certainly wasn't something that I was doing formally or with any particular endpoint in mind. It was more of just a general examination.
Q. Did you print any articles that you discovered through your PubMed or other personal research?
A. I don't think I did, no.
Q. Did you at some point weigh the evidence that you had seen in order to research a conclusion on the safety of talcum powder products?
MR. FROST: Objection.
A. As I said, it wasn't a formal assessment. There wasn't really a conclusion. It was a general look at the body of science to see what the body of science said on the matter.
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BY MR. SOILEAU:
Q. For what purpose?
A. For personal interest.
Q. Well, was it simple curiosity, or were you also interested in the safety of the talcum powder products?
A. Well, again, it is curiosity. As a toxicologist, I have just a general interest in toxicological matters and toxicological research, and I certainly -- whenever I see things in media or other sources, I like to see what the scientific basis is for the statements that are being made to see if the science supports it or doesn't support it.
Q. And what conclusion did you reach?
MR. FROST: Objection.
A. Again, you know, there wasn't really an end goal of making a conclusion. You know, as I said, when I reviewed the scientific literature, I certainly didn't see the body of science showing a strong evidence to support anything in regards to the

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statements that I was -- you know, the general nuances that were being put forth in the media.
BY MR. SOILEAU:
Q. Did you rely on that personal research in deciding to use Johnson & Johnson's baby powder on your newborn?
MR. FROST: Objection.
A. I certainly used my -- you know, I certainly looked at the scientific knowledge and the body of science in regards to using Johnson & Johnson baby powder on my newborn. I mean, I knew the literature before I had my son.
BY MR. SOILEAU:
Q. What had you heard through news articles that triggered this personal research?
A. Goodness, it was so many years ago. I remember seeing something about a large case that had just come to an end regarding talcum powder and ovarian cancer.
Q. Did you reach an opinion based on your personal research that the use of

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talcum powder products was safe?
MR. FROST: Objection.
A. Well, again, at the time, I was doing it more for personal interest as opposed to any general conclusions, but as I said, my impression at the time was that the scientific evidence did not support the statements being made in the media.
BY MR. SOILEAU:
Q. But did you determine that the use of talcum powder products based on your own personal research was safe?
MR. FROST: Objection.
A. Again, that wasn't the specific end goal. There wasn't a specific end goal in my assessment of the scientific literature. I had seen the media statements that had piqued my interest. I did a general look into the body of science to see if the body of science supported the statements being made by the media.
BY MR. SOILEAU:
Q. So you heard some statements or read some statements in the media, fair?

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A. Yes.
Q. Those did not come from scientists, did they?
A. I don't remember at this point who was making the statements, but they were in the general media.
Q. Okay. Certainly they did not appear in scientific literature, fair?
A. The ones that piqued my interest were -- I don't believe -- no, I don't believe they were in the scientific literature.
Q. So then you turned as a toxicologist to the scientific literature to conduct this personal research in response to the media reports, correct?
MR. FROST: Objection.
A. As I said, not necessarily in response. The media reports piqued my interest, and so I looked at the body of science regarding it.
BY MR. SOILEAU:
Q. All right. You had not looked at the body of science regarding talcum

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powder products before you came into contact with these media reports; is that correct?
A. Not that I recall.
Q. Okay. So you became aware of the media reports, and then that piqued your interest and you turned to the scientific literature?
A. Regarding the role of talcum powder in ovarian cancer, yes.
Q. Okay. And when you turned to the literature, you turned to the scientific literature, correct?
A. That's correct.
Q. And when you looked at the scientific literature, you looked at that literature as a toxicologist, right?
MR. FROST: Objection.
A. I applied my -- excuse me -- training and expertise as a toxicologist in reading the literature. I don't know what it means to look at it as a tox- -- as a toxicologist.
BY MR. SOILEAU:
Q. Well, the Casarett exhibit we

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looked at before said that toxicology offers a way to understand, prevent, mitigate and treat the potentially harmful consequences of substances.

Do you recall that? You have it there.

A. Yes, I recall us discussing it, yes.

Q. I mean, isn't that what you were doing, looking at scientific literature to understand and potentially prevent any potentially harmful consequences of this particular product, talcum powder?

MR. FROST: Objection.

A. As I said, I was looking at the scientific literature to see whether the scientific evidence was supportive of what had been -- what I had read or seen in the media at the time.

BY MR. SOILEAU:

Q. Okay. Do you know as we sit here today whether Johnson & Johnson employs toxicologists?

A. I don't know who Johnson &

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Johnson employs.

Q. Well, of course, to be clear, you understand I'm not asking you for the names of particular toxicologists, right?

A. Yes, I understand.

Q. Okay. But do you have any -- a sense of whether they have their own scientists on staff, including toxicologists?

MR. FROST: Objection.

A. Again, I don't know who Johnson & Johnson specifically employs on their staff.

BY MR. SOILEAU:

Q. Would you expect that Johnson & Johnson would have, as part of its team, scientists, including toxicologists with training and background similar to yours?

MR. FROST: Objection.

A. Again, I don't know who Johnson & Johnson hires or directly employs, so I can't speak to what their training and experience would be.

BY MR. SOILEAU:

Q. I understand that, Doctor. I'm

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really just asking about your expectation, and if you don't know and you don't have one, you can certainly tell me that.

But would you have, based on your education, experience and background, any expectation whether a company like Johnson & Johnson would employ scientists, including toxicologists, to look at any hazards that may be included within their products?

MR. FROST: Objection.

A. I'm sorry. That...

BY MR. SOILEAU:

Q. Too long?

A. Yes.

Q. All right. You have education, experience, training and other background in the area of toxicology, right?

A. Yes, that's correct.

Q. And we've talked a little bit about what toxicology is. I'm simply asking you as a professional: Would you expect that a company like Johnson & Johnson that sells talcum powder products among its product

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lines would have, within its company, scientists, including scientists with a background similar to yours, to assess any hazard their products might present?

MR. FROST: Objection.

A. And again, I don't know who Johnson & Johnson hires directly or how they do their manufacturing process or testing processes.

BY MR. SOILEAU:

Q. From your perspective, do you have any expertise in the regulatory area?

A. So in regards to my training and expertise in toxicology, that involves a wide range of subcategories, including regulatory aspects of toxicology and regulations regarding toxicological aspects.

Q. Okay. Let me show you an additional exhibit.

(Comments off the stenographic record.)

MR. SOILEAU: I'm sorry, Doctor, I have a -- here we go. I have a natural ability to lose items,

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and I lost our exhibit stickers for a moment, but I have found them.

(Whereupon, Deposition Exhibit Tuttle-6, 21 C.F.R. 740, was marked for identification.)

BY MR. SOILEAU:

Q. Let me show you a document I'll mark as Tuttle Exhibit 6. It's a two-page document. And I just want to know if this is something that, first, is familiar to you.

And not necessarily the document, Doctor, but to be specific, this document shows a copy of a regulation from the Code of Federal Regulations Title 21, 740, Section 740.1.

Are you familiar with this section from the Code of Federal Regulations?

A. I would need to look at it. Looks like it's a very brief paragraph. I'm familiar with the Code of Federal Regulations and the general documents.

Q. Well, let me ask you this: This document says, and specifically subpart (a) of Section 740.1 says that the label of a

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cosmetic product shall bear a warning statement whenever necessary or appropriate to prevent a health hazard that may be associated with the product.

Do you agree with that?

MR. FROST: Objection to form.

A. Well, this is one subpart of the entire part 740 that was -- was not provided for review to put the additional context for this, so I can't -- without knowing more or being able to read the entire regulations regarding this for context, I can't agree or disagree.

BY MR. SOILEAU:

Q. Okay. Very well.

Let me ask you this: As a toxicologist with your training and background and the experience that you bring to this project, do you believe that a manufacturer has an obligation to identify hazards that may be associated with its products?

MR. FROST: Objection.

A. It depends.

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BY MR. SOILEAU:

Q. Well, could you agree that good practices of a company include an obligation to identify hazards that may be associated with the products that it presents for sale to citizen consumers?

A. Well, again --

MR. FROST: Objection.

A. -- that's a very broad statement. It depends. You have to look at specifics, specifically what questions you're wanting to answer, what the regulations say regarding individual products and assessments and things. So, you know, that's overly broad to just say yes or no.

BY MR. SOILEAU:

Q. Okay. One of the things I want to understand today as we talk is the scope of your expertise and the issues or topics that you might address through your testimony, and I know you told me that you have some contact with regulatory matters.

I'm really not asking you to interpret or list regulations right now. I'm

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just asking you as a toxicologist with your training and background, do you have an understanding about good practices for a company like Johnson & Johnson?

MR. FROST: Objection.

A. Well, as I said, as part of my training and experience in working in toxicology, I work with regulatory guidelines and regulatory standards. You know, I can't speak to what good practices would be. You know, that's kind of a general term.

As I said, I spend a lot of time reading regulations and looking at regulations, but regarding good practices for a company or anything like that, I would need to look at the regulations.

BY MR. SOILEAU:

Q. Do you ever work directly with companies, companies like Johnson & Johnson, who present for sale to citizen consumers products like talcum powder products that are at issue in this case?

MR. FROST: Objection.

A. I have worked with companies in

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regards to product information and product -- and regulations regarding products. I don't think that I've been involved with any that were direct-to-consumer products.
BY MR. SOILEAU:
Q. What about warning? Have you ever worked on a warning? For example, a company brings you some item that may be a retail product, it may be used in industry, asked you to assist that company in preparing an appropriate warning associated with the product.
Ever done that?
A. So I've worked with companies in the generation of material safety data sheets under the Global Harmonization Standard as well as any warnings or labels required by the Global Harmonization Standard.
Q. What companies have you worked with on the issue of warnings?
MR. FROST: Objection to the extent it's not confidential.
A. I'm trying to --

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BY MR. SOILEAU:
Q. Yeah. Go ahead. If there's some issue of confidentiality, I'm not asking about the product, the warning or any of the product data that you might have reviewed.
I'm just asking you: What companies have you provided input on warnings to?
A. I don't know that I can recall all of them. As you said, I don't know if there's anything regarding confidentiality. I have done some work with Nucor Steel previously.
Q. Well, can we agree that an initial step is to determine whether any hazard exists in the particular product or item that you are looking at and considering?
Did that question make sense to you?
A. Could you clarify it for me, please?
Q. Sure.
Can we agree that an initial step in determining an appropriate warning

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for a product is to identify from the perspective of a toxicologist any potential hazard the product might present?
A. So that's a little broad. In regards to hazards and labeling, the Global Harmonization Standard puts forth some guidelines regarding that, but you assess the body of science, the scientific literature and the information available. It would, again, depend on the product, what was being manufactured, what we were looking at, what was the question.
Q. You say in part it would depend on the product. I mean, from a fundamental level, I mean, isn't it true that you need to know if there are any hazards presented by a product before you consider what, if any, warnings are appropriate or necessary?
A. Well, it's not just quite as simple as that. Like I said, you have to look at the scientific literature regarding what the science shows. There's some guidelines set forth in the Global Harmonization Standard regarding how hazard

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statements and labels are used based on the body of science and the scientific evidence.
Q. Have you at any time, in this project or otherwise, taken any steps or undertaken any work as a toxicologist to identify any hazards associated with talcum powder products?
MR. FROST: Objection.
A. Well, as I said in this particular case, I was asked to assess the body of science regarding a -- whether the body of science supports the evidence for a causal association between talcum powder applied perineally and ovarian cancer.
BY MR. SOILEAU:
Q. Do you recognize the words "hazard assessment"?
A. Yes.
Q. What do those words mean to you as a toxicologist?
A. Well, I don't know that I have a personal view. They are defined in various regulations and guidelines. I don't know that I personally have a definition. I would

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refer to those for a specific definition.
Q. Is hazard assessment something that you as a toxicologist have been trained to perform?
A. Yes, I have performed hazard assessments.
Q. Is there a methodology that you are familiar with that is used for the performance of hazard assessments?
A. I believe I touched on that briefly in my report, though not in depth. It would depend. There are some guidelines in the regulatory agencies in regards to hazard assessment.
Q. There's one than more -- I'm sorry, I tangled that up. I apologize.
There's more than one methodology out there can be used appropriately by toxicologists to conduct a hazard assessment, correct?
MR. FROST: Objection.
A. I would have to look at the methodologies in question. I'd have to look at the guidelines to see what methodologies

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you're discussing, if there are differences in them or anything else.
BY MR. SOILEAU:
Q. Okay. Very well.
Doctor, is there, however, a particular methodology that you use and employ and consider appropriate for hazard assessment?
A. Well, as I said, it would depend. I'd have to go look at the regulations to see what is put forth in those regulations regarding the methodologies for hazard assessment. I can't just speak to it generally.
Q. Okay. Have you ever performed a hazard assessment?
A. It's been a while, but yes.
Q. Okay. Are you familiar with the NRC risk assessment methodology?
A. Vaguely, yes.
Q. Is it generally accepted from your perspective as a toxicologist as one of the methodologies that might be used for hazard or risk assessment by a toxicologist?

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MR. FROST: Objection.
A. Well, again, regarding risk assessment, I would need to look at the methodologies you're discussing specifically so I could actually refer to them. You referred to multiple methodologies. I would need to see them to be able to compare them and see what the guidelines and regulations say.
BY MR. SOILEAU:
Q. Right. But I'm not asking you now about all of the methodologies. I was just asking you if you recognized the NRC risk assessment methodology as one of the methodologies that a toxicologist might use to perform an assessment?
A. And as I said, I'd need to see the methodology to be able to answer that appropriately.
Q. Okay. As we sit here today, you would not have any criticism of the use of the NRC risk assessment methodology for risk or hazard assessment, would you?
MR. FROST: Objection.

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A. Again, without having the methodology in front of me so I could look at it specifically, I couldn't say one way or the other.
BY MR. SOILEAU:
Q. Fair enough.
Did you make any -- well, let's ask this first.
Did you have any conversations, any discussions with any representatives of Johnson & Johnson, other than the attorneys retained to defend Johnson & Johnson? Any representatives of Johnson & Johnson other than their retained defense attorneys?
A. No, I have not.
Q. Did you take any steps to find out if the folks at Johnson & Johnson thought that their talcum powder products were safe?
MR. FROST: Objection.
A. No, I did not.
BY MR. SOILEAU:
Q. Have you assumed in your work during this case that the folks at Johnson & Johnson believed that their talcum powder

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products are safe?
MR. FROST: Objection.
A. I was not asked to look at what Johnson & Johnson thought. I was asked to examine the body of science and the scientific evidence.
BY MR. SOILEAU:
Q. Can you -- strike that.
Wouldn't you expect that Johnson & Johnson would have its own body of scientific evidence and scientific information addressing talcum powder products?
MR. FROST: Objection.
BY MR. SOILEAU:
Q. Its talcum powder products?
A. Again, I was asked to assess the scientific literature and the body of evidence regarding a -- whether the evidence supported a causal association between perineal talcum powder application and ovarian cancer.
It wouldn't really -- what Johnson & Johnson thought or anything

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wouldn't play a role in the assessment of the scientific literature.
Q. Was the scientific literature, the body of evidence that you considered, was it provided to you, or did you take any steps to generate or assimilate that body of scientific literature and evidence?
A. No, the literature was not provided to me. I did the literature reviews myself as well as -- we have an information specialist at CTEH that helps with interlibrary loans and PubMed searches and things of that nature.
Q. An information specialist?
A. Yes.
Q. Who is that?
A. Her name is Samantha Nation.
Q. Was it your intention in gathering scientific literature and evidence to be thorough and complete?
A. It was certainly my intent to look at the body of science as a whole.
Q. And when you say as a whole, the entire available body of science? Would

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that be fair, to say that you intended to look at the entire available body of science as a whole?
MR. FROST: Objection.
A. Well, the entire body of science can -- you know, when you're searching PubMed and searching Google Scholar or looking at these sources, you're sifting through thousands to millions of scientific articles on a range of topics. So we certainly attempt to be comprehensive and address the topic as put forth in the scientific literature thoroughly.
But when you're dealing with that many publications and that sheer amount of data, you know, there's no guarantee that you have found literally every applicable publication in the scientific literature.
BY MR. SOILEAU:
Q. That makes sense.
Can we agree that in gathering the scientific literature, it is your intention and you aspire to gather all of the relevant scientific information?

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MR. FROST: Objection.
A. We certainly attempt to gather as much, you know, applicable and, you know, the scientific evidence that discusses the question we're trying to answer as best we can.
BY MR. SOILEAU:
Q. But for the reasons you outlined a moment ago, it can be hard to find studies on a particular question or issue, fair?
A. Well, I said it can certainly be hard to say that you have found every article that has ever been published on any given topic.
Q. Wouldn't you expect that Johnson & Johnson would be an excellent source for relevant scientific literature and evidence on the question of any relationship between perineal application of talcum powder products and ovarian cancer?
MR. FROST: Objection.
A. I don't know what Johnson & Johnson maintains as far as scientific

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literature or what they would do as far as being a source for that kind of thing. I went to my established databases and did the searches myself to find the literature.
BY MR. SOILEAU:
Q. Do you know how long Johnson & Johnson as a company has been marketing to the public talcum powder products?
MR. FROST: Objection.
A. No, I do not.
BY MR. SOILEAU:
Q. Okay. And you said you don't know what Johnson & Johnson maintains. Did you ask at any point?
A. No, I did not.
Q. So as we sit here today, you do not know whether Johnson & Johnson believes its talcum powder products are safe?
MR. FROST: Objection.
A. Well, as I said, I was asked to look at the body of science and the scientific evidence regarding whether perineal talc exposure was causally associated with ovarian cancer, regarding --

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I'm not -- you know, I am not aware of Johnson & Johnson, their opinions or thoughts regarding anything of that nature.
BY MR. SOILEAU:
Q. Okay. That's fair.
So Johnson & Johnson's position or what Johnson & Johnson thinks about the safety of its talcum powder products is simply a question that you were not asked to look at and have not looked at, fair?
A. Again, I looked at the body of science, I looked at what the scientific evidence shows. What Johnson & Johnson thought or does, you know, is separate from that. That's not something that I would be looking at when looking at the scientific evidence.
Q. But as we sit here today, Doctor, you don't know what scientific evidence or what literature Johnson & Johnson has itself gathered or might have in its possession, do you?
MR. FROST: Objection.
A. As I said, I went to the

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peer-reviewed scientific literature. I do not know, as I said, what Johnson & Johnson has done or maintained.
BY MR. SOILEAU:
Q. Okay. Were you provided with Johnson & Johnson documents that might speak to any relationship between perineal application of talcum powder products and ovarian cancer?
MR. FROST: Objection.
A. I am not -- as I said, I'm not aware of what Johnson & Johnson has. The materials that were provided to me are listed in my report under the materials reviewed and relied upon.
BY MR. SOILEAU:
Q. All right. Look at page 2 of your report, if you would, Section 3.0.
Do you have that page, Doctor?
A. Yes, I do.
Q. You see that 3.0 has a number of bulleted items?
A. Yes.
Q. The last one says, quote "Other

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Provided Documents," closed quotes.
Do you see that?
A. Yes, I do.
Q. Are there any other provided documents that you have not identified for us as we sit here today?
A. Let's see.
Q. To be fair, I do have your supplemental list that we received this week, and we may look at that today.
A. Yes.
Q. And I understand that there's a supplemental list you have produced. We'll attach it perhaps later. It's not included in Exhibit 1, your report, but certainly those things that are on the supplemental list have been identified to us. So that's understood.
A. Okay.
Q. I just want to make sure that as to Other Provided Documents under Section 3.0 of your report, there's not anything that's not been identified to us as we sit here today.

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Does that make sense?
A. Yes. I'm just reviewing the list to see if there's anything that comes to my mind that was provided --
Q. Sure.
A. -- that may have been...
Q. This is my opportunity to talk to you and to be fair and complete. I would not want to be in a courtroom later and hear that, well, aha, under Section 3.0 on page 2, I said other provided documents and there were some other documents we never identified for you, and we meant to reference that through that generic language.
A. Sure.
Q. There's nothing like that, is there?
A. No, I don't believe so. I believe everything that was provided is cited here, and then the scientific literature in my references, I'm trying to think if there was any instance where we were unable to pull an article that we had found, you know, in our PubMed searches that we went to the

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attorneys to get a copy of, but I don't think that we had to do that.
Q. Okay.
A. But if we had, it would have been cited in the references earlier in the report.
Q. Exactly. Right. If you had run into that obstacle, it still would have made -- that is, that literature that was not immediately available to you would have made its way into the reference list that begins at page 72 of your report, correct?
I didn't mean for you to check the page.
A. Yes.
Q. I'm just trying to identify the reference list.
You put in the reference list, as supplemented by the list we got this week, all of the materials that you're relying on, fair?
A. Yes.
Q. Okay. Let's look at pages 4 and 5 of your report, if you will.

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At pages 4 and 5 of your report, you list and discuss the nine Hill aspects, don't you?
A. Yes, I list and briefly discuss the nine Hill -- excuse me -- the nine Hill criteria.
Q. Okay. Very well. Let me show you a document that I will mark as Tuttle Exhibit 7.
(Whereupon, Deposition Exhibit Tuttle-7, 1965 Hill Publication, was marked for identification.)
BY MR. SOILEAU:
Q. Do you recognize Tuttle Exhibit 7?
A. Yes, I do.
Q. What is Tuttle Exhibit 7?
A. So this is the 1965 Hill article entitled The Environment and Disease: Association or Causation, that I cite in my report.
Q. Do you consider this sort of a seminal paper?
A. It certainly is, as I state in

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my report, one of the -- well, I mean, obviously it's now the criteria is known as the Hill criteria, and that, with the other reference I cite, is some of the literature that established methodology for establishing whether an association is merely correlative or if it is causative.
Q. You said again, known as the Hill criteria. Do you know whether Sir Austin Bradford Hill ever used the word "criteria" in this paper?
A. Not off the top of my head, no, I don't know if he used that word specifically. I would need to look through it specifically for that particular word.
Q. All right. You do say that Hill -- this I see at page 5 of your report.
You say that Hill concludes that these nine viewpoints should be studied noting none of the viewpoints provides indisputable evidence, correct?
A. Yes, I state that Sir Hill stressed that the nine viewpoints should be examined as a whole and that no -- as I said,

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no one viewpoint alone could provide indisputable evidence.
Q. But that's not all he said, is it?
MR. FROST: Objection.
BY MR. SOILEAU:
Q. Well, let me ask you: Didn't he go on to say that while none of his nine viewpoints can bring indisputable evidence for or against the cause and effect hypothesis, none can be required as a sine qua non?
A. I'm sorry, can you refer me to where you are in this article, please?
Q. Sure. Do you recognize that from the Hill paper, the language I just read?
A. I'd like to be able to see it on the page, if you don't mind.
Q. And I'm glad to show it to you, but I wanted to ask if you recognized it first before we look at it in the paper.
Do you?
A. Briefly. As I said, I'd need

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to see it in the actual article so I can kind of see the entire sentence as a whole.
Q. Okay. Let me -- let's look at it. I'm looking at page -- it says 11 at the top left and 299 to the top right. I believe this comes out of an occupational medicine textbook and that's why there are two different page numbers.
Do you see page 11 at the top left?
A. Yes, I do.
Q. And you see a paragraph that begins with here then are nine different viewpoints from all of which we could study -- I'm sorry, we should study association before we cry causation.
Do you see that?
A. Yes, I see that paragraph.
Q. And Sir Bradford Hill goes on to say: What I do not believe, and this has been suggested, is that we can usefully lay down some hard-and-fast rules of evidence that must be obeyed before we accept cause and effect.

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Did I read that correctly?
A. Yes, you did. And as I said, and he says it here, the nine different viewpoints must all be studied before you can make a determination regarding causal association, which is what I believe I said, you can't pull one viewpoint, as we were saying earlier, and provide from one viewpoint indisputable evidence.
Q. Right. And the word "must" in the quote I just read, that is emphasized in the original paper from Sir Bradford Hill, isn't it; that is, what I do not believe is that we can usefully lay down some hard-and-fast rules of evidence that must be obeyed? He emphasized the word "must."
A. Yes, it is italicized here in this report.
Q. All right. The next sentence includes the "none of the nine viewpoints bring indisputable evidence" that you quote from in your paper, your report, correct?
A. Let me double-check my quote to make sure it's --

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Q. Sure.
A. Yes.
Q. But you only used the first part of this sentence which reads, quote, "None of my nine viewpoints can bring indisputable evidence."
You don't use the rest of the sentence, do you, in your report?
A. In my report, I specifically just quote the indisputable evidence words, since I was taking that directly from his report.
Q. Well, from a scientific approach, Doctor, do you think it's fair to quote Sir Bradford Hill on the indisputable evidence that may or may not be offered by any of his viewpoints and omit the language where he says "none can be required as a sine qua non?
MR. FROST: Objection.
BY MR. SOILEAU:
Q. Do you think that's good practice?
MR. FROST: Objection.

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A. Well, again, I think if you -- as I said, I took "indisputable evidence" directly from his report, which is why it is put in quotations and cited for accuracy.
BY MR. SOILEAU:
Q. I understand that.
A. But again, as I said, you can't take any single of these viewpoints put forth by Hill and be -- as it says, bring indisputable evidence. You can't take any single criteria in a vacuum and make a determination regarding a causal association. All nine should be studied -- should be studied, which is what he states at the beginning of that paragraph.
Q. Certainly. And none can be required, fair?
MR. FROST: Objection.
A. As I said, he states at the beginning that all of which they should study before establishing a causal opinion.
BY MR. SOILEAU:
Q. Doesn't Sir Austin Bradford Hill also teach us that none can be required

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before we determine cause and effect?
MR. FROST: Objection.
A. Again, as I said before, you can't take one in a vacuum and use one criteria to either prove or dis- -- the Hill criteria. You have to take all nine and look at the context of all nine as a whole.
BY MR. SOILEAU:
Q. You cannot take one criteria to prove -- you said to either prove or. Isn't it more complete and correct to say you can't take one in a vacuum, one criteria, to either prove or disprove causation?
MR. FROST: Objection.
A. No, I stopped myself because that's inaccurate. Scientifically, you can't prove a negative.
BY MR. SOILEAU:
Q. Well, okay. I'm sorry, I feel like I cut you off. I didn't mean to. Go ahead.
A. Thank you.
So you can't take one criteria in a vacuum, you can't take one criteria out

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of the nine without assessing the other nine and assessing the body of science as a whole, which is what Sir Hill is stating here when he says the nine different viewpoints from all -- all of which we should study association before we cry causation.
Q. What does sine qua non mean? Do you know? How is your Latin?
A. My Latin is very rusty. I took Latin in undergrad but that was a while ago.
Q. You don't know what it means?
A. Not off the top of my head, no.
Q. Very well.
Are you familiar with a textbook called Modern Epidemiology?
A. Maybe vaguely.
Q. Do you know who the authors are?
A. No, I do not.
Q. Do you know whether there is a textbook that is universally accepted in the United States as a leading textbook on epidemiology?
MR. FROST: Objection.

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A. No, I'm not aware of that.
BY MR. SOILEAU:
Q. Have you heard the name Sander Greenland?
A. No, I don't believe I have.
Q. Have you heard the name Kenneth Rothman?
A. Maybe vaguely, but I'm not familiar with him.
Q. Okay. Do you own a copy of Modern Epidemiology?
A. I don't believe so, no.
Q. Do you know whether you ever studied a course that required the use of the textbook Modern Epidemiology in your various educational endeavors?
A. I certainly studied epidemiology as part of my training and expertise in toxicology. I don't recall having a textbook on epidemiology solely.
Q. All right. Let me show you an excerpt from a textbook called Modern Epidemiology, Third Edition. I will mark this as Exhibit 8.

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(Whereupon, Deposition Exhibit Tuttle-8, Excerpt from Modern Toxicology Third Edition, was marked for identification.)

BY MR. SOILEAU:

Q. I don't suppose it does, but let me just ask. Does the first page of Tuttle Exhibit 8, which is the cover of the textbook, happen to trigger any recollection?

A. No, it does not.

Q. All right. Then let's turn to the excerpt that I have from page 26 of Modern Epidemiology, and I have here just a section called or labeled Causal Criteria. It's under Section 1 of Basic Concepts of the textbook Modern Epidemiology.

And I'd like you to look. You see in the last paragraph of this document a reference to Hill. You see that?

A. Yes, I do.

Q. And just for context, if you go one paragraph up, you will see a reference as well to the viewpoints proposed by Sir Austin Bradford Hill dated 1965.

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A. Yes, I see that.

Q. And if we come back down to the final paragraph, you see that the nine viewpoints are included in the middle of that paragraph?

A. Yes, I see them listed.

Q. Okay. We're talking about the same paper that is now Tuttle Exhibit 7, correct?

A. I believe so. I would need to look at the references to be specific. I don't know if Sir Hill published anything else in that year.

Q. Okay. Very well.

Can we agree that the nine viewpoints that are listed in the final paragraph of Tuttle Exhibit 8 are identical to the nine viewpoints that you included in your report beginning at pages 4 and 5?

Do you need to check them?

A. I'm just looking to make sure that the wording is the same.

Q. Absolutely. Do that.

A. Yes, I believe so.

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Q. Okay. I want to read to you the end of this paragraph from the Modern Epidemiology textbook. It says: Hill emphasized that causal inferences cannot be based on a set of rules, condemned emphasis on statistical significance testing, and recognized the importance of many other factors in decision-making, citing Phillips and Goodman, 2004. Nonetheless, the misguided but popular view that his considerations should be used as criteria for causal inference makes it necessary to examine them in detail.

Do you agree or disagree with that summary statement about Hill?

MR. FROST: Objection.

A. Well --

BY MR. SOILEAU:

Q. Or I'm sorry, or if you're not able to offer a comment, certainly that can be the answer. It need not be agree/disagree. It can be I don't have an answer or position.

Go ahead, I'm sorry.

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A. As I said, I'm not familiar with this textbook. The statement regarding Hill does not cite Hill's work; it cites another article that I'm not familiar with and don't know the title. I would need to see that article, the basis for that statement, before I could provide any discussion on it.

Q. Okay. And in fairness to you, you're not really familiar with these authors, Rothman, Greenland and Lash, to react to the fact that it's their textbook, fair?

A. No. As I said, I'm not familiar with this textbook and I'm not familiar with the authors.

Q. Okay. Very well.

Let's focus for a few minutes, if we could -- I'm going to check.

MR. SOILEAU: Everybody doing okay time-wise, break-wise, my reporter? All right. If someone needs a break, let me know. Otherwise, we'll keep going.

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THE WITNESS: I'm nearing the time I will need a break, just a very brief one.
MR. SOILEAU: You want to go ahead and take it now? It's a stop spot. Let's do that. Let's take a quick break. We'll go off the record.
THE VIDEOGRAPHER: Going off record, 10:24 a.m.
(Recess taken, 10:24 a.m. to 10:34 a.m.)
THE VIDEOGRAPHER: We're back on the record at 10:34 a.m.
BY MR. SOILEAU:
Q. Okay. Doctor, we had a short break. Are you ready to proceed?
A. Yes, I am.
Q. Very well.
Let's focus on one of the Hugh -- that's going to be tough because none of us have looked at Hugh.
Let's look at one of the Hill viewpoints, okay? Plausibility.
You say at page 30 of your

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report, in part: The plausibility of a relationship -- let me let you get there. Do you see it?
A. Yes, I'm there.
Q. Okay. On page 30 you have a subheading for one of the Hill viewpoints and that is plausibility, which I believe is number 6, correct?
A. Of the --
Q. Of the nine.
A. I don't apply a number to them, but it looks like it's number six as far as the list.
Q. I tell you what. Look at page 5 of your report for a moment. Do you see plausibility there?
A. Yes.
Q. It's number 6?
A. Yes, it's listed as number 6.
Q. Very good. Let's go back to page 30 now of your report, if we could. And under the subheading Plausibility, you say in part, and I will quote: The plausibility of a relationship between talc exposure and

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ovarian cancer relies solely on the proximity of talc particles to the ovaries, and the proposed migration to the ovaries.
Did I read that sentence correctly?
A. Yes, you read that sentence correctly. I believe I discuss -- I state in the next sentence I discuss it in greater detail later in the report.
Q. Sure.
When you say in that sentence, Doctor, the proximity of talc particles to the ovaries, what do you mean?
A. So I'm referring to the hypothesis that perineal application of talcum powder can migrate from the perineum to the ovaries.
Q. Do you agree that perineal application of talcum powder will put talc particles and talcum powder in proximity to the ovaries?
MR. FROST: Objection.
A. Again, as I discuss in my report, the scientific evidence has not

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established that perineal application of talcum powder will allow talcum particles to migrate from the genital area to the ovaries.
BY MR. SOILEAU:
Q. Okay. So the issue you have is with the idea of migration, fair?
A. What I address in my report is the hypothesis that the perineal application of talcum powder will allow talc particles to migrate from the genital area to the ovaries.
Q. How did the talcum particles -- let me resay that.
How do the talc particles come to be in close proximity to the ovaries?
MR. FROST: Objection.
BY MR. SOILEAU:
Q. Do you have an understanding?
A. Well, as I said for -- for -- as I'm discussing in this report and as I discuss later in the report, I am examining the scientific evidence for the hypothesis that external application of talcum powder to the genital area will allow talc particles to migrate through the female reproductive tract

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to the ovaries.
Q. Why do you say talc particles as opposed to talcum powder?
A. Because that's what I refer to specifically in my report and in assessing some of the scientific literature, they're looking for talc particles in the ovaries, and so that's why I used the term "talc particles."
Q. Okay. So your statement here that plausibility relies solely on something, it's really that the plausibility of a relationship relies solely on the proposed migration theory? Is that a fair summary of what you're telling us there?
MR. FROST: Objection.
BY MR. SOILEAU:
Q. You say solely and then I see talc particles in proximity to the ovaries and proposed migration. I see more than one thing. I just want to understand what you mean when you say relies solely on. Relies solely on what?
A. So in relation to the -- again,

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the assessment of the scientific literature regarding perineal application of talcum powder and ovarian cancer there is the proposed migration that the external perineal application of talcum powder will cause talc particles to migrate throughout the female reproductive tract to the ovaries.
And secondarily to that is that the presence of talc particles at the ovaries is -- I'm sorry, I'm trying to make sure I'm speaking very clearly -- that the presence of talc particles at the ovaries is sufficient to -- or insufficient to establish a causal association with ovarian cancer.
Q. Would the presence of talc particles at the ovaries be important from your perspective as a toxicologist?
MR. FROST: Objection.
A. Well, again, not assessing necessarily the migration, the presence of a material at an organ or at a tissue does not mean that it will exert an adverse effect.
BY MR. SOILEAU:
Q. Well, it doesn't mean that it

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will, is what you're telling me?
MR. FROST: Objection.
A. I'm saying that the mere presence of a particle or chemical or anything when looking at toxicology, the mere presence of a material does not mean that the material will cause an adverse effect on the tissue or organ that it's located at.
BY MR. SOILEAU:
Q. But it might?
MR. FROST: Objection.
A. Again, it depends. You have to look at the scientific evidence, you have to look at the scientific literature, you have to look at dose and frequency of exposures and all those different parameters.
BY MR. SOILEAU:
Q. Okay. I'll tell you what. We'll come back to that issue in a moment.
I meant to ask you: How many hours have you spent with the J&J attorneys thus far during this project?
A. I couldn't give you an exact number. I would estimate we have probably

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spent between 20 and 25 hours together.
Q. Okay. That includes the time yesterday?
A. Yes.
Q. Let me show you a couple of exhibits that I will mark separately as Tuttle Exhibit 9 and Tuttle Exhibit 10.
(Whereupon, Deposition Exhibit Tuttle-9, CTEH Billing Summary, was marked for identification.)
(Whereupon, Deposition Exhibit Tuttle-10, CTEH Billing Summary, was marked for identification.)
BY MR. SOILEAU:
Q. Take a look at these two documents and identify them for me.
MR. SOILEAU: And for the purposes of counsel --
MR. FROST: I was going to say, do you mind telling me which one is 9 and which one is 10?
MR. SOILEAU: Exactly.
BY MR. SOILEAU:
Q. Why don't you help us because

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you have them there as I've already marked them.
A. So these -- I'm sorry.
Q. Yes.
MR. SOILEAU: Before you do that, we have different invoice numbers. And, Mr. Frost, if you can look over at Exhibit 9 and if you'll look at the invoice number and tell us which one is Exhibit 9 and which one is Exhibit 10 so I don't get it wrong.
MR. FROST: Exhibit 9 is 316304.
MR. SOILEAU: Very good. And then I will deduce that the other one is 10.
MR. FROST: I'm going to make the same assumption.
BY MR. SOILEAU:
Q. Very good.
Okay, Doctor, and I apologize for interrupting you. Go ahead. You were going to tell me what these are.
A. These are invoices provided by

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CTEH regarding my work on the Johnson & Johnson Daubert challenge.
Q. The Johnson & Johnson Daubert challenge.
Is this, to your knowledge, the only file that CTEH has opened for Johnson & Johnson?
A. To my knowledge, yes. I don't know what other individuals in the company are working on, but to my knowledge, yes.
Q. Well, fair. But -- I think we're together.
As we sit here, you don't have any knowledge and you're not aware of anyone else working at CTEH on a Johnson & Johnson talcum powder project other than the team that's worked with you on this Johnson & Johnson Daubert challenge; is that fair?
A. I'm sorry, can you restate that? I got a little confused there towards the end.
Q. Yeah. I'll come back to it.
How do I know who all at CTEH has worked on this project?

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A. So all of our time is billed and documented in our invoices.
Q. Right. But how do I know whose time I'm looking at?
A. The first page is just a summary of the hours that were billed that particular day, and in the subsequent pages, they break that down by employee.
Q. Right. But you see that my copy is redacted.
A. Yes, I do.
Q. Is there any way for me to know the names of the folks at CTEH who have worked on this Johnson & Johnson Daubert challenge project?
A. It would be -- I could probably tell you most of them. We have a team that supports -- I have a team that has supported me in my drafting of my report in this case, and that's their additional time that would be itemized in this invoice.
Q. All right. Do that for me, if you would, if you would give me names and titles or job positions. I know you gave us

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one already. You gave us Samantha Nation?
A. Nation.
Q. And she was an information specialist?
A. Yes.
Q. Do you happen to know her rate?
A. Not off the top of my head, no.
Q. Okay. Who else has worked on this project at CTEH?
A. So in addition to Samantha, we have also had a health scientist, Dana Cubanski.
Q. How do you spell her last name?
A. C-U-B-A-N-S-K-I.
Q. Very well.
And what does that mean, health scientist? How is she degreed, for example?
A. She -- and I don't remember specifically. She has a master's in science. I don't remember her exact field for her degree.
Q. All right. Who else?
A. In addition to Dana, Dr. Scott Malm, M-A-L-M, who is a toxicologist.

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Q. What role does he play on this?
A. He just provided some support for me reviewing my report, you know, looking at presentation, you know, typos, you know, checking, double-checking the report for me.
Q. Sort of a peer review? Not formally, but sort of?
A. Not really a peer review. Mainly just assisting with looking at formatting, presentation issues, making -- trying to help make sure when you're writing a report and dealing with something of this size, making sure there's no typos or making sure that I don't use inappropriate punctuation or my sentences end and I don't just kind of jump off onto another thought process or things like that.
Q. But he is a Ph.D. toxicologist?
A. Yes, he is.
Q. If I were comparing the two of you on some sort of company chart, is one superior or -- to the other, overseeing the other, or are you sort of on parallel platforms?

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A. I don't oversee Dr. Malm. He has been with the company for a shorter period of time than I, but beyond that, there's no --
Q. Y'all are just colleagues?
A. Yes.
Q. Who else?
A. Dr. Michael Reilly.
Q. Last name?
A. R-E-I-L-L-Y, I believe.
Q. All right. And his position?
A. He is also a toxicologist.
Q. Simply another colleague to you and Dr. Malm?
A. Yes.
Q. And he is a Ph.D. toxicologist?
A. Yes, he is.
Q. And what role did Dr. Reilly have?
A. He did the same type of support work for me as Dr. Malm.
Q. Any others at CTEH who have worked on this Johnson & Johnson Daubert challenge project?

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A. I think all that's left is some support staff who assisted with organizing the file of materials, you know, with the references and things like that.
Q. Is their time billed as well?
A. I believe so, yes.
Q. Okay. Their work would have been clerical or administrative, as opposed to substantive?
A. Yes.
Q. They were not reviewing your report or your work or your opinions and offering substantive comment, were they?
A. No, they were not. And as I said earlier, you know, those who did review my report were reviewing it for, you know, clarity, for presentation issues, for punctuation, formatting, things of that nature.
Q. Right. I was just really referring to that last group of people who are thus far unidentified and understanding their role as more administrative or clerical. That's fair, right?

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A. Yes, that's fair.
Q. And I didn't mean to include, just so that we are together, Dr. Malm or the other persons at CTEH who may have worked with you.
Is there any way for me to look at Exhibit 9 and Exhibit 10 and know, for example, how many hours you have?
A. With the numbers redacted --
Q. I'm sorry, the what?
A. I'm sorry, not the numbers redacted. With the names and times redacted, generally speaking, looking at Exhibit 9, I believe right here at the page 3 of 4 and page 4, where the hourly rate put forth by CTEH is listed as $305 an hour, that would be my time that I billed for this particular invoice.
Q. Would Dr. Malm, for example, have the same rate?
A. No, he would not.
Q. What is his rate?
A. I don't know specifically.
Q. Are you the only person at CTEH

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who worked on the Johnson & Johnson Daubert challenge project who billed at $305 an hour?
A. I believe so, yes.
Q. So to your understanding, I can at least look for the itemized pages, look at the rate, and when I see $305 an hour, you would suggest I could assume correctly that that is you?
A. Yes, I believe so.
Q. And as to the other rates shown, I could correctly assume at least that it is someone other than you, someone at CTEH other than you, fair?
A. Yes, that's correct.
Q. Okay. Let me show you something we made reference to earlier and I'll make part of our record, marked as Exhibit 11.
(Whereupon, Deposition Exhibit Tuttle-11, Tuttle Supplemental Materials Reviewed and Considered, was marked for identification.)
BY MR. SOILEAU:
Q. It's a one-page document.

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Do you recognize this document, Doctor?
A. Yes, I do.
Q. And this is the supplemental materials reviewed and considered?
A. Yes, it is.
Q. And how was it determined to your knowledge what supplemental materials you would review and consider?
MR. FROST: Objection.
BY MR. SOILEAU:
Q. I'm just asking you -- how about this so that we don't step into anything we're not supposed to step into.
Who determined what supplemental materials you were to look at?
A. I did. As far as, I believe, all the materials provided here were ones that I read or reviewed.
Q. Did you -- I'm sorry, go ahead.
A. No. And so they were ones that I chose to read and review.
Q. Okay. Did you have a greater library of documents available and select

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these from that broader library?
A. I believe the attorneys provided all of the defense expert reports that are related to the MDL litigation. These are the subset that I read specifically or reviewed specifically. I don't believe I received all of the depositions, but I think I received -- but the ones that I reviewed are provided here.
Q. Okay. There are 12 items listed on Tuttle Exhibit 11?
A. Yes.
Q. And I believe each one of the 12 is either a deposition or an expert report, with the exception of item 11. Agree?
A. Yes, that's correct.
Q. How did 11, the Taher, T-A-H-E-R, the Taher paper, come to be among the supplemental materials reviewed and considered?
MR. FROST: Objection.
A. It was discussed in the Health Canada draft assessment that I

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mentioned in my report, and it was discussed in, I believe, at least one if not other -- other depositions, and I did not have a copy as it's unpublished, so I can't access it in the peer-reviewed literature.
BY MR. SOILEAU:
Q. Okay. You had, in preparing your original report, looked at Health Canada, the draft assessment from Health Canada?
A. Yes, I cite it in my report.
Q. Okay. And so when you signed your report, you would have been aware of the Taher paper, fair?
A. As I said, I saw that it was cited in the Health Canada draft assessment, but as it's unpublished, it was inaccessible in the peer-reviewed scientific literature.
Q. Okay. And you had seen that before you signed your report, fair?
A. The Health Canada draft assessment, yes.
Q. Right. And the reference to Taher, the unpublished paper?

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A. Yes, I saw the reference in the Health Canada report.
Q. Okay. Had you requested the Taher paper before you signed your report?
A. I don't believe so, no.
Q. What caused you to decide to request the Taher paper after you signed your report?
A. As I said, it was unpublished, so that's why it would not have been included in my review of the scientific literature. It was included later because, as I said, I saw that it had been discussed more in detail, and so -- and since I couldn't receive a copy of it through normal scientific literature means, the attorneys provided me a copy.
Q. You said you saw that it had been discussed more in detail. Where?
A. I don't recall specifically, but in some of these supplemental materials that I reviewed.
Q. Some of the supplemental materials listed on Tuttle Exhibit 11?

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A. Yes.
Q. During your preparation for your deposition testimony, have you been videotaped?
MR. FROST: Objection.
She can answer.
MR. SOILEAU: Okay.
A. No, I have not.
BY MR. SOILEAU:
Q. Okay. Let's turn to the issue of migration. You say at page 57 of your report, Section 11.5, that Dr. Plunkett's migration theory whereby talc particles supposedly migrate through the body either through perineal application or inhalation, and arrive at foreign tissues such as the ovaries, is severely flawed.
Did I read that correctly from your report? Did you find it in time?
A. I got to the page, but you were about halfway through the sentence.
Q. All right. Let's just see if we can agree on this.
At Section 11.5, and I think

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particularly in the heading, you do say that Dr. Plunkett's migration theory is, in your opinion, severely flawed, correct?
A. 11.5 does state Dr. Plunkett's theory of particle migration from the genital area to the ovary has not been accomplished in the scientific literature and is severely flawed.
Q. Very well.
Turn to page 60 of your report. You say in Section 12.2 of your report, referring to Dr. Zelikoff, that the opinion that talcum powder can reach the ovaries by means of migration through the female reproductive tract after perineal application is not scientifically sound, correct?
MR. FROST: Objection.
A. Can you --
BY MR. SOILEAU:
Q. Do you say that -- sure. I can help you find it if you need me to. I'm asking you if you labeled Dr. Zelikoff's opinion on migration as not scientifically sound.

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MR. FROST: Objection.
A. In the first paragraph under 12.2, I say: In her expert report, Dr. Zelikoff opines that talcum powder can reach the ovaries by means of migration through the female reproductive tract after perineal application as well as through inhalation. Neither opinion is scientifically sound.
BY MR. SOILEAU:
Q. Okay. So it is correct that you have labeled Dr. Zelikoff's opinion on migration as not scientifically sound and Dr. Plunkett's theory of migration as severely flawed, correct?
A. It's stated as not scientifically supported, as stated in my report.
Q. Are those things synonymous to you?
A. I'm sorry, what things?
Q. Not scientifically supported and unsound or severely flawed, are all of those three things synonymous for you in

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your -- in your -- when you use those words?
A. So in looking at Dr. Plunkett and Dr. Zelikoff in regards to migration, it's looking at the methodology applied and looking at the scientific evidence that supports or doesn't support their statements, and what I am saying here is that based on the scientific evidence, there's no scientific evidence that establishes that perineal application of talcum powder can migrate through the female reproductive tract and reach the ovaries.
Q. No scientific evidence to establish that perineal application of talcum powder can migrate to the ovaries; is that correct?
A. I believe I said no scientific evidence that supports that perineal application of talcum powder can migrate through the reproductive tract to the ovaries.
Q. Thank you. No scientific evidence that supports that perineal application of talcum powder can migrate.

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And what you're saying is, is that no scientific evidence to support this idea that after perineal application, the talcum powder can migrate through the reproductive tract to the ovaries?
A. As I said, there's no scientific evidence to support that perineal application of talcum powder will migrate from the genital area through the female reproductive tract to the ovaries.
Q. Right. I guess I was just understanding. I think it's clear the thing that migrates would be the talcum powder product; is that right?
You talk about perineal application of talcum powder and then you talk about migration from the genital area through the reproductive tract to the ovaries. What's migrating under this theory as you understand it?
A. Under the hypothesis as I understand it is that the talcum powder would migrate through the female reproductive tract, or as I state, we discussed earlier in

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regards to talc particles based on, you know, looking at some of the scientific literature that looks at talc particles.
Q. Very well.
Doctor, have you formed an opinion that migration of talcum powder to the ovaries after perineal application is not plausible?
MR. FROST: Objection.
A. So what I did was assess the body of science to see what the scientific evidence supports, and the scientific evidence does not support the hypothesis that perineal application of talcum powder can cause talcum powder to migrate from the genital area to the ovaries.
BY MR. SOILEAU:
Q. You brought up the issue of migration in your report, specifically under the topic of Hill's plausibility viewpoint, didn't you?
A. Yes, I did.
Q. Do you have an opinion on whether migration is a plausible explanation?

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MR. FROST: Objection.
A. Well, as I said, it's not about my opinions about what the scientific evidence shows, and the scientific evidence doesn't support that, that mechanism.
BY MR. SOILEAU:
Q. Have you conducted a thorough review of the available scientific evidence and then formed an opinion on migration based upon that review?
A. Well, as I said, I assessed the body of science, and as we discussed earlier, it's impossible to say out of the millions of scientific literature that I reviewed every single article in the realm of scientific literature, but I looked at the body of science and saw -- assessed it in the context of the Hill criteria and found that it does not support the hypothesis that perineal application of talcum powder can migrate from the genital area to the ovaries.
Q. What I understand you to be telling me is that you did not find scientific evidence to support the migration

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theory.
Have I understood you correctly?
A. Yes, I believe so. As I -- just to reiterate for clarity, in assessing the body of science, I found no scientific studies that supported the perineal application of talcum powder that could migrate from the genital area to the ovaries.
Q. In your evaluation of the scientific evidence, did you conclude that the plausibility viewpoint fails on that basis?
MR. FROST: Objection.
A. I would need to return back to get my exact wording regarding the plausibility on page 30.
BY MR. SOILEAU:
Q. Go ahead and do that, and I'll reset my question.
You had discussed with me earlier the nine viewpoints that Sir Austin Bradford Hill provided in his 1965 paper, correct?

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A. Yes.
Q. And you had stressed -- or said -- maybe stressed is unfair, but you had specifically said at some point that each of those nine viewpoints should be considered, correct?
A. Yes, that's accurate. I said we should look at all nine criteria as a whole for the context, and that you can't take one criteria in a vacuum.
Q. Right. But the application of Hill methodology would include a review of each of the viewpoints as part of the whole application of the methodology.
Do I have it right?
A. Yes, that's correct.
Q. Okay. And your paper includes the plausibility viewpoint, a discussion of plausibility?
A. Yes, it does.
Q. So did the scientific evidence that you looked at -- that you looked at as you reviewed the literature and the available scientific evidence satisfy or fail to

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satisfy plausibility under the Hill viewpoints?
A. So as I note in Section 30 and as I discuss elsewhere in my report, the scientific evidence -- there's no scientific evidence to support that migration theory that perineal application of talcum powder applied to the genital area can migrate through the female reproductive tract to the ovaries.
Q. Okay. And I'm sorry if you told me this before, but this issue of migration is one of the things that you reviewed as you did your overall literature search, correct?
A. Yes, that's correct.
Q. And to be complete, I understand you've told me that there are thousands of papers, the possibility of missing one is there, and I understand also that your review of literature was not limited to migration. That's fair as well?
A. Yes, that's accurate.
Q. Okay. I just wanted to make

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sure it included migration. I appreciate your patience with my questions.
In looking at the body of your report, I found three things that you listed in opposition to this theory of migration. One of them was gravity, is that correct, that you list gravity as a fact that stands in opposition to the migration theory?
MR. FROST: Objection.
A. I need to -- refer to me -- or sorry -- refer me specifically to where I discuss gravity. I do know that I mention gravity. I don't know that I present it as a fact in opposition of the migration theory.
As I said, I looked at the body of science and found the scientific evidence does not support the migration theory.
BY MR. SOILEAU:
Q. Okay. When you review a body of scientific literature in general, do you weigh available evidence as part of your methodology?
A. It depends. You know, I look at the body of science as a whole. In this

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particular case, you know, we -- I cite the available scientific literature as well as discussing issues that may be part of the study or the body -- you know, how the scientific study was performed.

But generally speaking, I try to look at all -- everything that's been published in the scientific literature as well as I can.

Q. Right. You don't start with the answer and look for literature to support the hypothesized answer only, right?

MR. FROST: Objection.

A. Exactly. I do not go to the scientific literature only looking for things that will support or disprove a particular hypothesis. I try to look at the body of science as a whole.

BY MR. SOILEAU:

Q. Right. And you've discussed that process with us some today and we may talk about it more, but as you gather the available literature, the body of scientific evidence, do you at some point in this

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process weigh the evidence to see what it tells you?

A. Well, again, we're speaking in generalities.

Q. Yes.

A. In the case of plausibility, as I said, the scientific evidence doesn't support the migration theory that perineal application can migrate to the ovaries.

Q. Okay. But you do recognize as part of a methodology in the scientific process for the analysis of a causal relationship the idea of weighing evidence, don't you?

MR. FROST: Objection.

A. Again, I'm familiar with the term "weight of evidence." I'm familiar with assessing the body of science. You know, as far as what I did here, I assessed the body of science as a whole, and as far as weighing individual things, that's very general. We'd have to probably get more specific into the body of science and what we were doing.

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BY MR. SOILEAU:

Q. Fair enough.

Doctor, during your work in this project and specifically the generation of your expert report, did you at any point weigh the evidence on the issue of migration?

MR. FROST: Objection.

A. As I said, the scientific evidence does not support the hypothesis that perineal application of talcum powder can cause talcum powder to migrate through the female reproductive tract to the ovaries.

BY MR. SOILEAU:

Q. Okay. There was really no evidence to weigh. Let me back up a step.

Sometimes when you look at an issue and you gather the available scientific literature, it is fair to say that at times you have competing literature, true?

A. Again -- again, that's very broad.

Q. Yes, ma'am.

A. We have to kind of look at a body of literature specifically for a

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topic --

Q. I understand.

A. -- and see what the science says.

Q. It was a general question, Doctor, and it's simply that when you gather the available literature, there are times for some issues where there is competing evidence on both sides of a question, fair?

A. Again, you have to look at the science as a whole as far as -- as competing. You know, again, you're speaking in generalities as regards to whether, you know, on a given topic science can have different, you know, viewpoints or different evidence.

Q. Sure.

A. The science is the science. You have to just look at the body of science as it stands and look at what it shows.

Q. I mean, that is the art of the scientific process, isn't it, gathering the evidence and looking at it and seeing what it teaches?

MR. FROST: Objection.

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A. I'm sorry, can you repeat the first part of that question?
BY MR. SOILEAU:
Q. Determination of a causal relationship of a substance like talcum powder is, for you as a toxicologist, different than engineering, isn't it?
MR. FROST: Objection.
A. I'm sorry. I don't think I understand the question.
BY MR. SOILEAU:
Q. Okay. I guess in my mind three engineers doing the same engineering calculation will come up with the exact same answer if they've done it appropriately and competently, but that scientists looking at evidence may have different thoughts or opinions even though they look at the same body of evidence.
Do you agree with that?
MR. FROST: Objection.
A. Again, we're speaking in generalities, but, no, if scientists are looking at the same body of science and

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applying the same methodology, they should arrive at the same conclusions.
BY MR. SOILEAU:
Q. Okay. Very well.
Is gravity in this context part of the body of evidence that you assembled when you examined the issue of migration of talcum powder through perineal application?
MR. FROST: Objection.
A. Well, as I said, you need to refer me to where I specifically address gravity.
BY MR. SOILEAU:
Q. Oh, I'm sorry.
A. I recall that I mention it, but as I said, I don't think I used it as a fact. I state that the scientific evidence does not support the migration theory.
Q. Sure. I'll point you to it.
A. Thank you.
MR. FROST: Do you want help?
BY MR. SOILEAU:
Q. Look at page 57.
MR. FROST: I did a word

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search.
MR. SOILEAU: I started to do that, but I made a pledge to myself that I would not open a computer and be further distracted. I think it was probably a good decision, but I appreciate your help. I don't mind you doing that. Saves us some time.
BY MR. SOILEAU:
Q. Do you have page 57?
A. I do.
Q. You say in the second paragraph of Section 11.5, which begins on page 57 of your report, I quote, "First, Dr. Plunkett's migration theory for perineal application would require talc to migrate upwards - against gravity."
And I'm stopping in mid-sentence. I just want to you ask about the gravity. We can continue in a moment, and I'll acknowledge that the report speaks for itself and continues.
But that's the gravity reference that I've seen.

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A. Yes, I see it.
Q. Do you have any scientific literature to support the idea that gravity is an answer or scientific evidence in opposition to the migration theory, or is this just sort of common sense?
A. Well, you know, again, you say that this is just one small portion of looks like at least four on that page, different points I discuss --
Q. That's true.
A. -- in relation to the migration theory.
Q. But if I ask you about them all at once I'll get in trouble, so I have to break them down and ask one at a time.
A. I understand.
Q. But that's fine. It's fair that there are several paragraphs and you say first, second, third and fourth on page 57. So I'm on first, and specifically, gravity.
What's the role of gravity in all this?
A. So again, gravity is just one

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small portion here, and, you know, it is part common sense. I can't provide you with a specific reference in regards to the gravity aspect. That mainly is just one part of the multiple things that I discuss here as far as the scientific literature not supporting the hypothesis that talcum powder can migrate from external perineal application to the ovaries.

Q. I see.

If I put a straw in this cup of water and the straw is narrow enough in its diameter, will the water always stay at the same level outside the straw and inside the straw or will it be different?

MR. FROST: Objection.

BY MR. SOILEAU:

Q. Or you don't know?

A. I don't know.

Q. Do you know whether gravity would keep the levels the same?

MR. FROST: Objection.

A. Again, I -- we can put a straw in the cup of water and perform the

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experiment.

BY MR. SOILEAU:

Q. Okay. Is there anything more you can tell me about your reference to gravity or scientific support for the reference to gravity in our discussion of this migration topic?

A. Well, as I said, I referred to it very generally in this sentence as part of an overall context regarding the other points that I used to address the migration theory. I know that there are others involved in this litigation that get into this in more detail than I do, so I would have to refer to them.

Q. Very well, Doctor.

In retrospect, do you think the inclusion of gravity in this discussion of migration of talcum powder products following perineal application is appropriate scientifically?

MR. FROST: Objection.

A. Well, again, it's one part of the different argument -- different things in examining the scientific evidence. You know,

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gravity is, again as I said, just one part of what I referred to here as far as looking at the scientific evidence and whether it can support the hypothesis of migration or not, and gravity is one of the arguments against the supportion of an upward migration.

BY MR. SOILEAU:

Q. I certainly understand it is one and only one of several points you make, but do you think, as we sit here today, the inclusion of gravity is appropriate in this discussion?

MR. FROST: Objection.

A. Well, again, gravity is a scientific concept and is part of --

BY MR. SOILEAU:

Q. Sure.

A. We have the law of gravity.

Q. Newton was right.

A. And so I included it here as one of several different things, and as I said, I think there are others who get into the nuances of migration theory much more in depth than I do.

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Q. Okay. You stand by the inclusion of gravity. How about that? Is that fair? In your report?

A. Yes, I included gravity in there and I do believe, as I said, as part of the scientific evidence and what I address elsewhere in my report it is part of that assessment.

Q. You say that there are others. Who are you talking about? Are you talking about other experts?

A. Yes, I believe there are other experts involved that get more into the science of the migration theory and discuss those nuances and stuff in more detail than I do.

Q. Do you consider yourself to be one of the experts on behalf of Johnson & Johnson possessing appropriate education, background, training and experience to offer opinions on the migration theory?

MR. FROST: Objection.

A. So as a toxicologist, toxicology is a very broad scientific field

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of study. It encompasses a large number of different scientific fields, including epidemiology, anatomy and physiology, chemistry, molecular biology, cancer biology, any number of different fields that all come under that umbrella in being able to research and understand how chemicals or materials can have a potential adverse effect on people or the environment or animals.
BY MR. SOILEAU:
Q. I've heard that. I appreciate that. I didn't cut you off, did I?
A. Go ahead.
Q. But do you feel comfortable discussing the migration theory within the boundaries of your expertise as you view -- as you view your expertise in this case?
MR. FROST: Objection.
A. Well, as I said, I reviewed the scientific literature regarding the migration theory and whether there's any scientific evidence to support the perineal application of talcum powder can reach the ovaries --
///

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BY MR. SOILEAU:
Q. Understood.
A. -- and found no scientific evidence to support that.
And as far as my training and expertise, I am -- you know, I review those types of studies as part of my ongoing work as a toxicologist. I'm not a gynecologist, so as I said, there are others involved that get into this in more detail than I do, and I would have to refer to their work and the data that they use in what they provide, but ultimately, I would look at the scientific literature and generate my own conclusions.
Q. Right. But, I mean, at home, I see my sink and the plumbing and I see water and I figure out there's a leak, but if it comes to who's going to figure out why it's leaking and fixing it, it's going to be a plumber; it's not me.
So I'm just trying to figure out, although you've looked at the literature, is this migration issue -- are you a plumber? Are you able to speak to

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migration or should we turn to some of these others to discuss that issue?
MR. FROST: Objection.
BY MR. SOILEAU:
Q. I just want to know your perspective.
A. No. And as I said, I'll try to be a little clearer. Maybe I'm not being very clear.
As part of my training and expertise in toxicology, you know, anatomy and physiology and molecular biology and things like that play -- are an important part of the overall science of toxicology.
So that being said, I have reviewed scientific literature regarding whether it supports the migration theory or not, and I have addressed it in my report and summarized it in my report.
Q. Yes.
A. I am aware that there are others involved in this litigation that, you know, focus on the migration theory much more specifically than I do and get into more

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detail.
Q. Okay.
A. And I would certainly refer to their work, but I would always ultimately go back to the science in forming my own opinions.
Q. I just want to make sure you're comfortable speaking with me today about migration as an issue in this case, staying within your own view of the boundaries or limits of your expertise.
So are you comfortable talking about migration with me today?
MR. FROST: Objection.
A. As I said, I will certainly -- I address migration in my report and discuss it in my report. I'm happy to discuss what I've included in my report if --
BY MR. SOILEAU:
Q. Okay.
A. -- you know, if in the toxicological implications and methodologies that I use here as far as the scientific evidence.

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Q. I want to ask one other question about that. You think -- I think you said refer to others, but it almost feels like you mean defer.
Do you defer to other experts on the migration?
MR. FROST: Objection.
BY MR. SOILEAU:
Q. Or just you would refer to their opinions too?
MR. FROST: Objection.
BY MR. SOILEAU:
Q. I feel like that was an unclear question. Did you follow it or you want me to kind of resay it?
MR. FROST: Objection.
A. You can clarify for me, please.
BY MR. SOILEAU:
Q. You used the word "refer" in talking about others, and as I understand it, other experts for Johnson & Johnson. Would you defer to those other experts on the issue of migration?
MR. FROST: Objection.

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A. Again, I would refer, and I -- that is correct. I would refer to their reports and things, but I would ultimately go to the science they cite and the basis for their reports in forming my own opinions.
BY MR. SOILEAU:
Q. Very well.
Let's turn back to page 57, where we were a moment ago, still under that second paragraph that begins with the words "First, Dr. Plunkett's migration theory."
Let me know when you're there.
A. Yes, I'm there.
Q. We read through against gravity and stopped. I want to go a little more, and I'll start at the beginning for context.
"First, Dr. Plunkett's migration theory for perineal application would require talc to migrate upwards - against gravity and the downward flow of bodily fluids in the female reproductive tract," dash.
Did I read that portion correctly?

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A. Yes, you read that portion correctly. This sentence does continue.
Q. Right. As I said before, it does continue. I want to acknowledge that and certainly the report will speak more fully for itself.
But I want to focus first on these words, "downward flow of bodily fluids." What role or what significance, if any, do you place in the downward flow of fluids in the context of migration of talcum powder following perineal application?
A. Well, again, similar to gravity, this is just one small nu- -- seven or eight words taken out of the whole page on where I look at the -- Dr. Plunkett's theory of particle migration, and it's in conjunction with the statement around gravity.
This is, again, just one small portion of it, where we -- and we move on to look at, you know, the scientific literature and scientific evidence that does not support that the particles applied perineally can

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migrate to the ovaries.
So again, it's just one part of the evidence that doesn't support the hypothesis.
Q. Okay. Is there any scientific evidence or literature cited in your report, Doctor, that supports the relevance of this downward flow of bodily fluids in the context of an analysis of migration?
A. I don't believe I cite anything in that particular sentence. You know, I -- again, I didn't get into great detail regarding the downward flow of bodily fluids, the female reproductive tract and the fluids generated there. They have their own defense mechanisms against the introduction of foreign bodies and things like that.
Again, I think that others in the -- involved in this litigation get into much more detail regarding that than I do. I'm speaking very generally here before I move into, you know, the complete argument, which I -- as we said, has, I believe, four different addressings here, but looking at

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the scientific literature as a whole.
Q. Do the ovaries have the ability to clear foreign particles?
A. I don't know.
Q. We're outside of your area now?
A. Yes, I have not researched that. I could -- would certainly be happy to research it, but it's not something I researched in this litigation.
Q. Separate from researching it or reviewing available literature, is it something -- is it a question that you believe falls within -- falls properly within your expertise, your education, training and expertise as a toxicologist?
MR. FROST: Objection.
A. Well, again, as I said before, you know, toxicology covers a wide range of sciences, and the potential effect of adverse health effects in the body is definitely in the realm of toxicology and study of research.
I did not research ovaries and their ability to clear materials. That's not

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something I specifically looked at in this litigation. It's something that I would certainly -- could research and look at the scientific evidence, but I didn't do it in this case.
BY MR. SOILEAU:
Q. Wouldn't you think that would be important?
MR. FROST: Objection.
A. Well, again, as I state in the evidence that we put forward here and I think we discussed it earlier, one, scientific evidence doesn't suppor[redacted] the talcum powder applied perineally can migrate to the ovaries, but secondarily, we discussed earlier that the presence of a chemical or particle or something at an organ does not necessarily mean that an adverse health effect will occur. You need more information.
So as I said, as far as looking at the scientific evidence, there's no evidence to support that it would migrate to begin with.

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BY MR. SOILEAU:
Q. Does the respiratory have the -- I'm sorry.
Does the respiratory system have the ability to clear foreign particles?
MR. FROST: Objection.
A. The respiratory system?
BY MR. SOILEAU:
Q. Yes.
A. Again, that's not something I address in my report.
Q. No, it's not.
Do you know, as we sit here today?
MR. FROST: Objection.
A. Generally speaking, and as I said, I don't address that specifically in my report, but the human body has -- has many defense mechanisms for removing materials from -- from its -- from the body.
BY MR. SOILEAU:
Q. What defense mechanisms do the ovaries have?
MR. FROST: Objection.

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A. Again, I haven't researched that specifically to address it. As I said, the human body has many defense mechanisms.
BY MR. SOILEAU:
Q. Okay. Let me show you the next exhibit, which I will mark as Tuttle Exhibit 12.
(Whereupon, Deposition Exhibit Tuttle-12, Excerpt from IARC Monograph 93, was marked for identification.)
BY MR. SOILEAU:
Q. Do you recognize, Doctor, the exhibit that I have presented to you?
A. Let me -- I'm trying to keep my report in order.
Q. Sure.
A. Yes, I do.
Q. What is this?
A. This is the 2010 International Agency for Research on Cancer monograph that includes, among -- talc as well as titanium dioxide and carbon black.
Q. It is cited in your report?

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A. Yes, it is.
Q. You have a quote at page 57, same page we were at earlier. Let me let you turn to that. It's still Section 11.5, the section that discusses Dr. Plunkett and her, as you describe in your report, theory of particle migration.
You say that IARC has concluded that the evidence for retrograde transport of talc to the ovaries in normal women is weak.
Do you see that?
A. Yes, I see where I state that in my report.
Q. All right. And you actually quote it. You have quote marks in your report for the words, "the evidence for retrograde transport of talc to the ovaries in normal women is weak," closed quotes, right?
A. Yes.
Q. And that is taken from the IARC Monograph No. 93, which is now Tuttle Exhibit 12?
A. That is the citation I believe.

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It says IARC 2010. I can refer to their reference list to be specific, but...
Q. Go ahead and look if you need to. Do you have it?
A. Yes.
Q. Okay.
A. And yes, the IARC 2010 reference is referring to the carbon black, titanium dioxide and talc monograph.
Q. Right. That monograph that is IARC Monograph No. 93 has three sections, one for carbon black, one for titanium dioxide and one for talc, right?
A. Yes, I believe so. It's a very large document, and we have a small subset, but I believe those are the three addressed specifically. I can't remember if this monograph discusses anything as a subset.
Q. Okay. That's fine. Why don't you turn to page 411. Take your time.
A. Okay.
Q. Do you see at the top of the page it says Talc?
A. Yes.

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Q. Okay. Now, in your quote, it says "the evidence for retrograde transport of talc to the ovaries in normal women."
Why does it say "normal women" in the quote you use that you cite and reference in your report?
A. I would need to refer to the IARC document and get -- pull out the original quote to pull the additional context for normal women.
Q. Do you know as we sit here right now without looking at the IARC monograph?
A. Off the top of my head, I can assume, but I would prefer not to assume.
Q. Sure. No, that sounds more of a guess than, you know -- an educated guess, but a guess, so let's not do that.
Did you independently review any literature on this issue that was referenced in or cited by IARC in its Monograph 93?
A. I would need to look at the references and compare them, but generally

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speaking, that is part of -- you know, when looking at some of these documents, you look at the data and the science and the reference they use to cite for what they state in their documents, or in this case, in the monograph.
Q. All right. I don't have your testimony right now. My realtime stopped, but let me see if I can understand better. There are two scenarios in my mind. One is you rely upon IARC and its monograph for the statement that is quoted in your report.
Another possibility is that you do not simply rely on the IARC monograph, but you independently pull, review and consider any literature cited by IARC in its monograph to form an independent scientific opinion, your own, that is, about what that literature might show.
Which of those two scenarios did you employ here as to IARC Monograph 93 and your discussion of IARC in the context of migration?
MR. FROST: Objection.
A. So again, the quotations and

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the wording as put forth in my report would be directed to the IARC monograph itself. Had that been taken from something else, it would have been cited to that.

With that being said, when reviewing the IARC monograph or any document like it that summarizes a body of science, you know, I try to go to the data that is -- they're reviewing and form my own opinions.

BY MR. SOILEAU:

Q. Okay. Let's take a look at the paragraph on 411 from which you took your quote.

A. And if I may, before you ask a question, we're getting close to the time that I'm going to need a break to go nurse.

Q. Okay. Let's -- if you need to stop, stop. I'm going to try to do two questions and stop, okay?

A. Okay.

Q. Lawyers are bad about estimating questions.

Let's look down here toward the bottom of 411. Where it says: On balance,

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the working group believed that the evidence for retrograde transport of talc to the ovaries in normal women is weak.

And I've highlighted it here on the page just to try to help you find it.

I just want to know, is this the source statement for the quotation in your report?

A. It appears to be, yes.

Q. Okay. All right. Then let me break a record and stop there and only ask one question. We'll take our break now. I promised you.

THE VIDEOGRAPHER: Going off the record at 11:34 a.m.

(Recess taken, 11:34 a.m. to 12:22 p.m.)

THE VIDEOGRAPHER: We're back on the record at 12:24 p.m.

BY MR. SOILEAU:

Q. Good afternoon, Doctor. Are you ready to proceed?

A. Yes, I am.

Q. Very good.

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Let's go back to page 411 of the monograph, and we had looked at the sentence on page 411 that I believe we recognized as the source sentence for the quote that you have in your report on page 57, which we discussed before the break. I want to direct you to some additional language in that same paragraph.

Do you see the third sentence that begins -- third sentence of the paragraph that begins: These have been conducted in women who were about to undergo gynecological surgery, most of whom had diseases or complications of the reproductive tract and organs that required surgery.

Did I read that correctly?

A. Yes, you read that correctly, but as you said, you took the third sentence down and it doesn't specify what "these" are. I think prior to that it says: Several studies have been conducted in women to assess potential retrograde movement of particles through the reproductive tract to the ovaries.

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Q. Okay. Let's go back to the beginning of the paragraph. That's fair.

It says, quote, "Perineal exposure to cosmetic talc in women is of concern because of its possible association with ovarian cancer," period.

And then next, the sentence you just referenced: Several studies have been conducted in women to assess potential retrograde movement of particles through the reproductive tract to the ovaries.

Have I read it correctly thus far?

A. Yes, you have.

Q. When it says retrograde movement, is that, in your view, synonymous with the issue of migration that we are discussing?

A. Well, again, it's referring to several different studies. As it says, as it goes through, it goes -- looking at the transport of talc to the ovaries where it says, you know, at the end, again, that the evidence in normal women is weak, and then

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also that evidence in animals with no retrograde transport of talc to the ovaries.
Q. Okay. Doctor, that was not my question. My question was simply: When IARC uses the word retrograde movement of particles, do you recognize that as being synonymous for our purposes with the theory of migration of talc particles?
A. Well, again, without seeing the specific studies that they're talking about, you know, I don't know what each individual study was looking at in regards to what they're defining as retrograde movement.
As I've said before, I -- the scientific evidence doesn't support a transport from external perineal application and so I'd have to look at each individual study that they're referencing here, which they don't cite at the end of this particular sentence.
Q. Right. You have not actually looked at any studies in connection with this IARC section, have you?
A. Well, as I said before, you

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know, there are no particular references listed right here in this paragraph for me to refer to.
Q. Right.
A. But that being said, as I said previously, I'm looking at the IARC or looking at any, you know, government or agency documents such as this, go through the references and look at the body of science that they refer to in looking at the body of science for scientific evidence.
So in order to discuss, you know, retrograde movement as stated here, I would need to go back and look at their references and look at the studies that they are citing.
Q. I think I follow you, but I want to ask: Do you know what retrograde movement of particles means as used here in this paragraph from which you have taken your quotation in your report?
A. Well, again, I don't have a specific definition. As it says here, it says movement of particles through the

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reproductive tract to the ovaries.
Q. Okay.
A. It does not specify from perineal application, doesn't specify from the external application, but merely through the reproductive tract to the ovaries.
Q. What are the first two words of the paragraph?
A. Of the paragraph, is perineal exposure, but the sentence that we're discussing just says several studies for potential retrograde movement.
Q. Okay. Let's continue in the paragraph. Does it go on to say: The findings reported in these studies may be confounded by the various levels of dysfunction in clearance from the female reproductive tract due to underlying pathologies.
Did I read that correctly?
A. Yes, you read that correctly.
Q. What does confounded mean as used in that sentence? Do you know?
A. Well, I think we discussed

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confounding generally speaking, but -- and again, we'd have to look at each individual study to see what the individuals say. Just saying that the findings reported in these studies may be confounded may be another term for it -- you know, may be affected or may -- you know, these other issues as far as this IARC says may be involved in the studies, but as I said, we have to look at the individual studies to discuss it specifically.
Q. Okay. You said we discussed confounding, but I don't think we did.
Do you know what confounding is?
MR. FROST: Objection.
A. As I said, I mentioned confounding in my report as far as talcum powder and ovarian cancer. In this particular sentence, they mention other things that may be involved in the studies that the IARC is citing, but without looking at those individual studies, I can't discuss, you know, these different things that they state may be confounding or may be involved

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in the studies that they're using.
BY MR. SOILEAU:
Q. Okay. I think I follow you. I believe you're telling me that without looking at the individual studies, you can't really be specific about how or why there may be confounding. You can't put any meat on those bones absent reference to the specific study; is that fair?
A. Right. This is a general statement about the studies in general, which again, are not cited in this particular paragraph, so we can't go to the references, which I don't think are in -- included here --
Q. Right.
A. -- for the basis why they say the studies may be confounded and state these issues.
Q. But can you offer a basic definition of the term "confounding" as it is used, not with reference to any particular study, but as a general principle? What does it mean that something may confound a study?

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MR. FROST: Objection.
BY MR. SOILEAU:
Q. Do you know?
A. Sorry, I'm trying -- I believe I talk a little bit about confounding in the causation section of my report, especially in regards to specific causation, when looking at confounding factors.
Q. Right. You understand here we're really focused on general causation, or do you understand that in this -- this Daubert proceeding that is referenced in your invoice as the Daubert challenge process?
You understand we're really looking at general causation as opposed to a particular woman?
A. Well, again, it is my understanding that we're looking at the scientific evidence to establish whether there is a causal association, which again, would be general causation --
Q. Right.
A. -- as far as what the scientific evidence supports.

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Q. But I'm not sure if you've told me what the word "confound" means in this context. Are you able to offer a definition of confound?
A. And any definition I would offer right now would be, you know, my interpretation or my kind of summation without having, you know, what I have in my report right in front of me, and I don't want to misquote what the scientific definition for confounding is.
Q. Yes, Doctor, I appreciate that.
Do you have an understanding of the principle of confounding within this context, the general principle?
MR. FROST: Objection.
A. Well, again, we're speaking in generalities, and without looking at these studies, confounding may mean something slightly different depending on the study and what the authors are doing as far as they state here, assessing potential retrograde movement of particles.
///

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BY MR. SOILEAU:
Q. You have used the word "confounded" before in your own writings, haven't you?
A. Yes, I have.
Q. When you use the word "confounded" in your own writings, what do you mean by that word?
A. Well, again, it would depend on the context. I'd have to, you know, as I said, I've certainly used it on numerous occasions, and -- but I would need to see the context of how I was using it to be able to accurately describe what I meant by it when I used it in a sentence.
Q. Let's do this. I'm going to show you another exhibit. I'll mark this as Tuttle Exhibit 13.
(Whereupon, Deposition Exhibit Tuttle-13, Egli and Newton Publication, was marked for identification.)
BY MR. SOILEAU:
Q. Perhaps it will save time.

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Could you -- I think you're going to. Go ahead and turn to the references that begin at page 72, I believe, of your report, and conveniently, it's alphabetized, so if you can go to the Es and see if this report by G.E. Egli, E-G-L-I, Dr. Egli is one of the reference materials that you considered in this matter?

A. It is not one of the references that I cite.

Q. Is it by chance familiar to you generally for whatever reason?

A. If I may, I'm just briefly looking at it.

Q. Let me direct you -- and you may do that, but I'm going to direct you to page 153, and there's some language under Discussion that says at page 153 of Tuttle Exhibit 13, the Egli paper, that this study indicates that in two cases under the conditions outlined, inert carbon particles placed in the posterior fornix of the vagina were found 28 and 34 minutes later in both tubes.

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Do you see that?

A. Yes, I see that. I'm not familiar with this article, but I note that it's dealing with, as you said, inert carbon particles and dealing with a vaginal application as opposed to a perineal.

Q. Okay. Do you know what the posterior fornix of the vagina is?

A. Not specifically, no, I don't know.

Q. Okay. Are you familiar with carbon particles generally as a substance or agent?

A. Yes, generally.

Q. Okay. Let me show you an additional paper. I'll mark this as Tuttle Exhibit 14.

(Whereupon, Deposition Exhibit Tuttle-14, 1971 Henderson et al Publication, was marked for identification.)

BY MR. SOILEAU:

Q. Tuttle Exhibit 14, the lead author is W.J. Henderson. It is a 1971

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paper. I think Egli was 1961. That was Tuttle Exhibit 13. So Exhibit 14 is Henderson 1971.

Can you look and see if that is referenced among the materials that you reviewed in your consideration of the scientific evidence and literature in this matter?

A. No, it is not.

Q. Could you turn to page 268.

A. If you don't mind, since this is something -- you know, that, as with the Egli study, that I have not seen, if you can give me just a moment to familiarize myself with it somewhat.

Q. Sure. I tell you what I'm going to do. I'm going to show you what I'm interested in for reference without asking you any questions about it, and then you can take some time if you need to to look at this paper, okay?

A. Thank you.

Q. So I'm looking at -- on page 3 of 7, which is 268, I've highlighted some

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language here that says: The talc particles were found deep within the tumor tissue. Talc particles were also found embedded within tumors of the cervix.

And second -- there are a number of photographs or images, perhaps is a better word, included in this paper. When you get into the discussion on page 271, the language that appears on the second column: There was good evidence for the presence of talc, often indistinguishable from anthophyllite asbestos within the ovarian tissue.

It also says, after the parenthetical: The talc particles were found localized deep within tumor tissues. And it continues.

Those are the two sections that I'm focused on in my review of this scientific paper.

A. Okay. And I apologize, you were moving a little too fast for me. Can I get you to direct me to where they are on the page on 268 and -- oh.

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Q. I'm going to loan you my highlighted copy of Tuttle Exhibit 14.
A. Thank you.
(Document review.)
BY MR. SOILEAU:
Q. As you're reviewing that paper, is it at all familiar to you?
A. As I said, no, I don't cite it in my report.
Q. Okay. I understand that. And your review thus far has not caused it -- it doesn't ring a bell. How about that?
A. Correct.
Q. I note in the summary of the Henderson '71 paper it says: An extraction-replication technique was used to examine tissue from patients with ovarian and cervical tumors. In both conditions talc particles were found deeply embedded within the tumor tissue. The close association of talc to the asbestos group of minerals is of interest.
That's on the first page.
Is there anything you wanted to

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add about this paper right now? I'm going to move to another one.
A. Certainly. The article appears to be the development of a method regarding the study of foreign particles within tissues, and I would not in -- after reading the snippets that you pulled from page 268 and 271, that the overall premise of this study was the development of a method, and they actually conclude at the end that although it is impossible to incriminate talc as a primary cause of carcinomatous changes within either the cervix or the ovary on the preliminary observations described here, and then they go on to say that further investigations are obviously required.
Q. Well, you just left out something, didn't you?
A. If you like, I can read it in its entirety.
Q. Didn't you omit the words: The possibility that talc may be related to other predisposing factors should not be disregarded?

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A. Yes, and we said "and further investigations are obviously required."
Q. Very well.
Let me show you a document that I'll mark as Tuttle Exhibit 15. This is a paper by Venter from 1979. And tell me first if it is included among your reference materials that list the studies and scientific evidence that you considered following your gathering process.
(Whereupon, Deposition Exhibit Tuttle-15, 1979 Venter et al Publication, was marked for identification.)
A. No, it is not.
BY MR. SOILEAU:
Q. Okay. I'm looking here on the first page. There's a reference to migration. It says: Such migration could well explain the etiological role of chemical substances in certain gynecological diseases.
Do you see that? The first page, second column.
A. Oh, okay.

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Q. Third sentence. The prior sentence says: Will a chemical substance deposited in the vagina later appear in the peritoneal cavity.
A. Yes, I see it.
Q. Okay. Let me show you another paper that I will mark as Tuttle Exhibit 16.
A. Well, if I may --
Q. Sure. You want to add something about that one?
A. -- I'm not familiar with this article, so I don't know what the premise is. I notice that what you said was deposited in the vagina, which is, again, different from perineal application is what we were discussing, and --
Q. Okay.
A. -- in this particular case. So I just wanted to point out that, again, this doesn't seem to be -- I haven't had time to review it or look at this report and see what the actual objectives of it were.
Q. I understand. And I want to allow you the opportunity to offer those

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comments. I understand that you've not reviewed it.
Really what I'm trying to do is verify that these studies that I have here are not studies that is -- that these studies that I have here are studies you have not considered in offering the opinions that you offer today.
Let me show you --
MR. FROST: Objection.
BY MR. SOILEAU:
Q. -- what I've marked as Tuttle Exhibit 16.
(Whereupon, Deposition Exhibit Tuttle-16, 1986 Henderson et al Publication, was marked for identification.)
BY MR. SOILEAU:
Q. When we say perineal, I mean, perineum, perineal -- perineal means involving the perineum; is that right?
A. When we refer to perineal application, generally we're referring to the application of talcum powder to the exterior

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genitalia.
Q. The perineum?
A. Yes.
Q. Does it include the opening of the vagina?
A. It includes the external opening.
Q. Right. Okay. I just want to make sure that that's clear, that we are talking about an area that includes the external opening of the vagina.
MR. FROST: Objection.
BY MR. SOILEAU:
Q. Is that right, to your understanding of the perineum?
A. Again, as I said, it involves the external genitalia of the female body.
Q. Including the vagina?
A. Yes, including the vagina.
Q. Okay. Let's turn to Exhibit 16, the Henderson paper from 1986. Could you tell me if that is included among the materials, evidence, scientific literature that you considered in connection

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with your work in this matter?
A. Again, it is not cited in my report.
Q. Do you see in this 1986 paper by Henderson and others, which we've marked as Tuttle Exhibit 16, on the first page, second paragraph of the introduction: Direct communication between the external environment and the peritoneal cavity exists in the female via her genital tract.
Do you see that statement, first of all?
A. Yes, I see that statement, but it's included in the introduction with no citations for it, so I don't know where they're basing -- you know, what that is based on.
Q. Okay. Do you agree or disagree with that statement as I read it?
MR. FROST: Objection.
A. As I just said, it's in the introduction. There's no reference cited to it. I don't know the basis for this statement, so I can't agree or disagree, and,

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you know, without reading this, having a moment to read this article or any of the articles that we've discussed, I -- you know, I can't discuss it in detail other than to say a cursory glance of all of these don't look at, as we were just saying, the perineal application of talc particles, but merely variations of either particles that are not talc or scenarios where either it's a method development and is not looking at how -- talc migration, period, other than just looking at a method development, and, you know, a lot of these are looking at vaginal introduction or something that doesn't -- that already kind of takes one step past the external application.
BY MR. SOILEAU:
Q. Okay. Do any of these papers that I have shown you thus far impact or move your opinion on migration?
MR. FROST: Objection. Sorry, I had food in my mouth. Objection.
MR. SOILEAU: Noted. We'll accept that.

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A. As I said before, I haven't had an opportunity to read these, look at their data, their scientific evidence. Some of these, I can't -- you know, I don't know what the purpose of these studies are or what they were being used for.
BY MR. SOILEAU:
Q. Very well.
Let me show you another article. This one I've marked as Tuttle Exhibit 17. It is by Kissler, K-I-S-S-L-E-R.
(Whereupon, Deposition Exhibit Tuttle-17, 2004 Kissler et al Publication, was marked for identification.)
BY MR. SOILEAU:
Q. Is this one included among the materials that you have reviewed, Doctor?
A. No, it is not.
Q. Look at the first page under the abstract, really the last sentence. It says, quote, "Directed uterine contractility and intact uterotubal transport function are considered necessary for intact sperm

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transport mainly due to the side bearing the dominant follicle to maximize fertility."
Do you see that sentence?
A. Yes, I see that, and this appears to, again, without having an opportunity to review it, this appears to be something that examines sperm transport, and...
Q. Right.
A. And again, uterine contractibility, but nothing about perineal application of talcum powder or talc particle migration from the external genitalia.
Q. Okay. What is directed uterine contractility, if you know?
MR. FROST: Objection.
A. I haven't read the article. I don't know.
BY MR. SOILEAU:
Q. Okay. And I'm -- to be clear, to be fair, I'm not asking you what meaning it has in the article. I'm asking you generally, what is directed uterine contractility? Do you know?

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MR. FROST: Objection.
A. Again, I -- no, I don't know. I haven't -- I haven't researched that.
BY MR. SOILEAU:
Q. All right. Let me show you what I will mark as Tuttle Exhibit 18.
(Whereupon, Deposition Exhibit Tuttle-18, 2004 Sj?sten et al Publication, was marked for identification.)
BY MR. SOILEAU:
Q. It is a 2004 paper, the lead author is Sj?sten, I think is the pronunciation. It is spelled S-J-O-S-T-E-N; the O has the double dots over it. We've exhausted my knowledge of that language. I believe it's the correct pronunciation.
Is this one included among the materials that you've reviewed, the one that I've marked as Tuttle Exhibit 18?
A. No, it is not.
Q. Look at the conclusion on page 1 of Tuttle Exhibit 18, the Sj?sten article. Conclusions: This study has

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pointed out a retrograde migration of starch also in humans after a gynecological examination with powdered gloves. Consequently, powder or other potentially harmful substance that can migrate from the vagina should be avoided.
Did I read that correctly?
MR. FROST: Objection.
A. Yes, you read that correctly, but again, with -- I haven't had a chance to review this. It appears, as you said, a gynecological examination, which would be, again -- it states it in your sentence, from the vagina as opposed to from the external genitalia, to the ovaries. And as you mentioned, it's looking at starch and not talcum powder.
But I would need some time to review this briefly to discuss it.
BY MR. SOILEAU:
Q. Do you recognize the journal that this article was apparently published in in 2004?
A. Not specifically, no.

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Q. Okay. It's Human Reproduction. That's not familiar to you, is it?
A. Not specifically, no.
Q. And, I'm sorry, you told me this was not included among the materials you reviewed, right?
MR. FROST: Objection.
A. That's correct.
BY MR. SOILEAU:
Q. I'm sorry if I asked that before.
Let me show you an additional study. I'll mark this one as Tuttle Exhibit 19.
(Whereupon, Deposition Exhibit Tuttle-19, 2019 McDonald et al Publication, was marked for identification.)
BY MR. SOILEAU:
Q. This is a 2019 paper, the lead author is McDonald. It was published, I believe, in Ultrastructural Pathology.
Is this paper included in the materials that you reviewed and considered in

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forming the opinions that you offer today, Doctor?
A. No, it is not.
Q. All right. If you would look to page 12 under Discussion, the second paragraph, where it states: Talc, when applied to the perineum, is believed to migrate to the upper genital tract, passing through the open tract to the fallopian tubes and eventually reaching the ovaries. End the quote.
Let me let you find that. I'm showing it on the screen, highlighted, but again, page 12, second paragraph of Discussion of Tuttle Exhibit 19.
Now, this statement is on point, isn't it?
MR. FROST: Objection.
BY MR. SOILEAU:
Q. Well, this statement -- let me be more specific. That's probably fair.
This statement is talking about talc and it's talking about talc applied to the perineum and it's talking about the

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perineal application of talc and the migration of talc into the upper genital tract through the fallopian tubes eventually and ultimately reaching the ovaries, right?
A. So again, you haven't given me an opportunity to read this article in its entirety, but I notice that after it makes that statement it cites two different residences, and if we go back and look --
Q. Sure.
A. -- it cites number 11, which is a Cramer study, which I don't believe we've discussed yet, but it's -- number 16 is the -- I believe it's the Henderson study we briefly touched on.
Q. Yes.
A. And again, none of the studies that you've shown me, I haven't had a chance to review them in detail, but none of them look at -- as this statement implies, talc when applied to perineum, to the external genitalia, and, you know, as it says here, is believed.
Q. Okay.

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A. So again, we're taking one sentence out of context, and these are all articles that I haven't had a chance to read in depth or look at the data or the information or even the objectives of these articles herein.
But none of them as stated have looked at the external perineal application of talcum powder and its potential or hypothetical migration.
Q. What do you mean you have not had a chance? "I've not had a chance to read." Why not?
A. Well, as I'm saying, as you've put them in front of me, you haven't given me an opportunity to look at them.
Q. Oh.
A. They weren't cited in my report.
Q. I understand. But there's no reason to think that they would not have been available to you prior to today, correct?
MR. FROST: Objection.
A. Well, as I said in cursory --

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as we've gone through these, there have -- without having a chance to actually look at them in detail, I can't speak to it specifically. We can go through each one, I'd be happy to.

In the case of the others not previously -- sorry, looking at the others we've previously discussed, again, none of them looked at external perineal application and talcum powder or they looked at particles that were not talcum powder, or in the case of some of them, they were just method developments and things. But I would need to be able to look at them specifically.

And then in the case of this McDonald one, actually, this one would not have been available via PubMed search judging by the article history on the front page saying that it was accepted in March of this year.

So again, I'd need to be able to read it and review it to discuss it.

BY MR. SOILEAU:

Q. Can we agree that the McDonald

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paper does address talc, application of talc to the perineum and migration of talc following perineal application to the ovaries?

MR. FROST: Objection.

BY MR. SOILEAU:

Q. That's what this is talking about, isn't it?

MR. FROST: Objection.

A. Again, I haven't had a chance to read anything on it yet except for the one sentence that we pulled that was referenced to two other publications, which as we were discussing, don't look at perineal application. I'm happy to take a minute to look at this so we can discuss it.

BY MR. SOILEAU:

Q. I just really wanted to know that this sentence is -- this sentence I'm quoting from the McDonald paper, which is Tuttle Exhibit 19, is talking about the thing you and I are talking about right now, migration of talc to the ovaries following perineal application.

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That sentence, whether you agree with it or disagree -- and I understand you've not seen this paper before today, but that topic sentence puts it squarely on point for the discussion we're having right now, doesn't it, Doctor?

MR. FROST: Objection.

A. Well, again, you've taken one sentence out of the second paragraph of the discussion -- of a discussion of an article that goes on for over two pages. It cites two references, neither of which support -- or well, excuse me, the one that we've looked at so far does not support the statement, and as it says, it just is believed.

So without being able to look at the entire document, you know, no, I can't speak to whether it is applicable to the external perineal application of talcum powder and migration or not.

BY MR. SOILEAU:

Q. Okay. Let's do this for a moment. Let's go back to Tuttle Exhibit 12. It's the excerpt from the IARC Monograph 93

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that you have before you. And by the way, you're correct, this isn't the entire monograph. I keep calling it the monograph, and I think you clarified that at some point, and I want to sort of stand in agreement with you. This is a section of that monograph.

But it starts with the discussion of mechanistic and other relevant data on talc and continues, beginning at page 391, for our exhibit, so...

I want you to turn to page 392. Do you recall when we were looking at the paragraph from which you took the quote that you used in your report, that is the IARC language that you quoted and included in your report, you made the point that they didn't show any studies there?

A. Yes, that --

Q. Remember on page 411?

A. Yes, that's correct.

Q. Okay. But now if we go back to page 392, they are talking about studies here, aren't they?

A. Yes, generally speaking, I see

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several citations throughout the text.
Q. Right. And if you go to the last paragraph on page 392, isn't it true that the first study referenced in this IARC monograph is Egli and Newton from 1961?
A. Yes, it is.
Q. And isn't that the same paper that I presented to you earlier, Egli and Newton, 1961, which is now Tuttle Exhibit 13?
A. Well, I would normally refer to the references of the monograph to confirm that. As I think I've said before, I don't know if Egli published anything else in that year.
Q. Well, read what it says. Egli and Newton, 1961 found that inert carbon particles deposited in the vagina in two of three patients traveled to the fallopian tubes in about 30 minutes.
Isn't that consistent with the Egli paper that we reviewed and that is Tuttle Exhibit 13?
MR. FROST: Objection.
A. Well, again, I wasn't -- I

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didn't get a chance to sit here and review the Egli paper. I do know that it looked at -- you know, is based on the title and what little chance I did get to look at it, it does involve inert carbon particles that were deposited in the vagina and looking at the fallopian tubes, but...
BY MR. SOILEAU:
Q. Well, let's do this. When I showed you the Egli paper from 1961 that we marked as Tuttle Exhibit 13, you reviewed your reference materials at my request, didn't you?
A. Yes, I did.
Q. And when you did that, you found no Egli paper from 1961, correct?
A. That's correct. I didn't cite it in my report.
Q. That's correct.
So regardless of how many papers Dr. Egli and Newton -- Drs. Egli and Newton published in 1961, if it's one or more than one, you didn't cite any of them in your reference materials. We can agree on that,

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can't we?
A. That's correct, I did not cite them in my report.
Q. So doesn't that tell us that you didn't look at an Egli and Newton paper from 1961?
A. Well, again, as I said, when looking at the -- what limited information I'm given, you know, this -- the Egli and Newton report, one, does not look at talc particles; two, does not like at perineal application; and three, in this particular statement as put forth by IARC, doesn't look at the ovaries but at the fallopian tubes.
So I am again, you know, not seeing anything based on what I've been able to review for this article that it would support the migration theory of perineal application of talcum powder and migration to the ovaries. So I don't see why I would have found it or cited it in my report.
Q. Okay, Doctor. But, no, that's not my point. My point -- let me clarify -- is you have used this monograph, Volume 93,

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and specifically some words on page 411 that you quote in your report to say that the working group believed that the evidence for retrograde transport of talc to the ovaries in normal women is weak.
You drew upon that and quoted it in your report, didn't you?
A. Yes, I quoted that in my report.
Q. Right. And I think we looked at it. It's at page 57 of your report, correct?
A. Yes, it's on page 57.
Q. And that Section 11.5 of your report, one of the sections that is quite critical of Dr. Plunkett, fair?
MR. FROST: Objection.
A. I'm not critical of Dr. Plunkett, merely of the methodology and -- the methodology used in Dr. Plunkett's report. And as I -- you know, as we've said before, this particular page has about four different topics that I address in addressing the theory of particle migration from the

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genital area to the ovaries.
BY MR. SOILEAU:
Q. Well, you did say in the topic sentence or title to Section 11.5 that Dr. Plunkett's theory of particle migration was severely flawed, didn't you?
MR. FROST: Objection.
A. As I -- I think I've said before, I state that the theory has not been established in the scientific literature and is severely flawed.
BY MR. SOILEAU:
Q. You did use the words "severely flawed" in referring to Dr. Plunkett and her discussion of particle migration, didn't you? Did you use those words, "severely flawed"?
A. I used severely flawed, but not in reference to Dr. Plunkett, but rather to the scientific -- you know, the theory of particle migration.
Q. Okay. Very well.
But my point is: In criticizing Dr. Plunkett, you quoted IARC, correct?

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MR. FROST: Objection.
BY MR. SOILEAU:
Q. Second paragraph, page 57 of your report, Section 11.5. You said: Indeed, IARC has concluded.
And then we get back to the quote.
A. Yes, I quote IARC there in that first -- that second paragraph under the heading 11.5. But again, I'm not critical of Dr. Plunkett, but merely the methodologies used.
Q. Yes. Well, speaking of methodology, though, Dr. Tuttle, we've gone now to the source document, the IARC Monograph 93, and we've found at 411 the source sentence for your quotation, fair?
A. Yes.
Q. And you told me, in looking together at 411, you did not see any studies cited, correct?
A. Yes, that's correct.
Q. But if we go back to 392, we do find studies, beginning with the Egli and

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Newton study, referenced by IARC in this discussion, fair?
A. In that particular paragraph. There are paragraphs before that that again cite several other articles, and there are other articles cited afterwards. We've only looked at the handful that you've presented to me that I haven't cited. We haven't gone through all the references in the IARC monograph to see which ones I cited and which ones I did not.
Q. Sure. That's fine. And my time is a bit limited, but we can agree that we know that this first study at the beginning of this paragraph, Egli and Newton, is cited by IARC, correct?
A. Yes, it is cited by IARC.
Q. And you did not look at it, did you?
MR. FROST: Objection.
A. Again, I did not cite it in my report. As I said previously, when looking at the IARC documents and looking at any of these documents, you look at the data and the

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references cited by them. I don't claim to have cited every reference that IARC cites in their monograph in my report.
BY MR. SOILEAU:
Q. But you quoted IARC for the conclusion, yet you didn't independently review the studies upon which their conclusion was based, did you?
MR. FROST: Objection.
A. Well, again, you're stating that, you handed me just a small handful of studies. We can go through all the references that they cite and then look also at my report and see which references they cite and which ones I cite and which ones they do and I do not.
As I said before, you know, you look at the data and you look at the scientific evidence, and the example you keep using of Egli and Newton, as I said before, one, is looking at inert carbon particles; two, looking at deposition in the vagina as opposed to external perineal application; three, as it says in two of three patients

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traveled to the fallopian tubes. It doesn't mention the ovaries specifically here.
BY MR. SOILEAU:
Q. Do you see the reference later in that same paragraph of page 392 of the IARC Monograph 93 to Henderson, et al, 1971?
A. Yes, I do.
Q. And I've presented you with a Henderson 1971 paper. It's now marked as Tuttle Exhibit 14, right?
A. I don't recall the exhibit number, but yes, you presented Henderson 1971 to me.
Q. Right. And you confirmed that you did not look at that paper either, right?
MR. FROST: Objection.
A. Again, I did not cite it in my report.
BY MR. SOILEAU:
Q. And if it's not cited, you didn't look at it. I mean, if you looked at it, you would have cited it, right?
A. Well, as I said, I would need to refamiliarize -- or I would need to go

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back and look at these articles that you are placing in front of me because I don't cite them in my report, which means -- meaning that I don't reference them.
That being said, you know, I need to look at them and look at what the basis for them is and understand, and then I can have a better ability to discuss why they weren't cited or why they weren't referenced in my report.
Q. Is it possible that you pulled the Henderson 1971 paper, looked at it, considered it, then set it aside and did not include it in your references of materials considered?
MR. FROST: Objection.
BY MR. SOILEAU:
Q. I just need to know if I can rely on the references as a complete listing of the scientific literature, the scientific evidence that was gathered and considered.
A. Well, as I've said before, in going through the scientific evidence, you're dealing with thousands upon millions of

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articles, so it would be -- I can't say specifically that I looked at it, read it and placed it to the side.
But that being said, I could very easily have looked at it and said this is not addressing external perineal application of talcum powder and migration to the ovaries; therefore, it's no evidence that supports the migration theory, and therefore didn't cite it in my report.
Q. And then you would kick it out of the materials considered?
A. Again, in the references, those are the references that I cite in my report, those are the references that I put into the report.
Q. That are helpful?
MR. FROST: Objection.
A. No, they're the references that I cite in the report.
BY MR. SOILEAU:
Q. Well, I understand there are thousands of papers out there. I'm just trying to understand what we have, because I

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may have misunderstood what your reference list is.
I would have assumed that if you did a search of the thousands of papers and you found Henderson 1971, and for the reasons you've told us, you didn't think it proved migration or even supported migration, you would nonetheless include it among the reference materials as something that you had identified and considered. Am I wrong?
MR. FROST: Objection.
A. Again, without giving me an opportunity to read Henderson, I can't speak to it specifically. What I'm saying is that in doing an assessment of science, I'd need to know, you know, what Henderson is actually looking at, what their tests are, what -- you know, and have an opportunity to actually review the article.
BY MR. SOILEAU:
Q. Right. But I might have thought you did that before you formed your opinion.
MR. FROST: Objection.

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BY MR. SOILEAU:
Q. Well, see, here's my problem. Based on your testimony this afternoon, I'm no longer comfortable that I know whether you looked at Henderson or not because it seems you're telling me you may have found the Henderson '71 paper, which is now Tuttle Exhibit 14, looked at it, concluded it did not support a relationship between perineal application and migration, and therefore set it aside so that it didn't make it into the reference list. Is that possible?
MR. FROST: Objection, misstates testimony.
BY MR. SOILEAU:
Q. I don't mean to state your testimony or rephrase your testimony. It's a question: Is it possible that in reviewing available literature, Henderson '71 was found, the same Henderson '71 that is now Tuttle Exhibit 14, that it was found, that you looked at it, and then you cast it away and therefore did not put it on the reference list. Is that possible?

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MR. FROST: Objection.
A. So I'll try to be very clear.
BY MR. SOILEAU:
Q. Good. That will help.
A. What I'm trying to explain is that in looking at the migration theory and the theory that external application of talc can migrate to the ovaries and looking at the scientific literature, and in my references or looking at IARC, I attempted to look at the scientific literature that actually examined the external application of talc and the potential for migration to the ovaries.
So if the -- if there's studies that look at other -- other things as we've talked about, vaginal insertion or, you know -- and again, I would need to look at these articles specifically to be able to speak to Henderson, which is the one we're discussing right now.
But generally speaking, there is no scientific evidence that has examined that external perineal application and migration through the female reproductive

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tract to the ovaries.
Q. If I understand your testimony, you're telling me that if you had come across Henderson, you would not have considered it relevant to the migration issue and therefore it would not have been listed; is that fair?
A. Again, you'd need to let me actually read Henderson and look at it to be able to say I don't cite it in my report, so I'm not familiar with the, you know, objectives or methods that were used in the study.
Q. Very well.
As we sit here today, last question on this. I want to move on. Last question on this, Doctor.
Do you know whether you ever saw Henderson or not in the context of this project, Henderson '71, Tuttle Exhibit 14, do you know whether you ever looked at it before or not?
MR. FROST: Objection.
A. I don't recall.
///

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BY MR. SOILEAU:
Q. Okay. Let me show you something that's been marked as Tuttle Exhibit 20.
(Whereupon, Deposition Exhibit Tuttle-20, Ovarian Cancer Prevention (PDR) Patient Information, was marked for identification.)
BY MR. SOILEAU:
Q. Do you know if you've seen this item previously?
A. I think I have seen it before, yes.
Q. Do you know who NCI is or what NCI is? It's not a trick question. It's on here. I should say: Do you recognize NCI as the National Cancer Institute? The print is very small, but if you look below the heading -- let me show you where. It's on there.
Do you see that?
A. Yes.
Q. Not to read, just to locate. Go ahead and look at your copy. So let me

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get a fair question to you, because I don't think we have one on the table at this moment.

Doctor, do you recognize the entity known as the National Cancer Institute?

A. Yes, I'm familiar with the National Cancer Institute.

Q. Do you recognize PDQ as something associated with the National Cancer Institute? You'll see that it appears here on the top title of this document, PDQ, and it has the R for restricted use.

Do you know what that is?

A. I don't think the R is restricted. I thought it was a registered.

Q. Oh, registered. I'm sorry, you're right. It's later in the day. You're doing better than me. That's why you're answering questions and I'm asking them.

Okay. Registered. So NCI has registered PDQ. Do you know what it means?

A. Not specifically. This appears to be a website with some patient

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information.

Q. Okay.

A. I do not know what PDQ stands for.

Q. Very well.

Look at page 3 of this item under Talc, and see what the National Cancer Institute has said about talc.

MR. FROST: Objection.

BY MR. SOILEAU:

Q. Talc. The use of talc may increase the risk of ovarian cancer. Talcum powder dusted on the perineum (the area between the vagina and the anus) may reach the ovaries by entering the vagina.

That's the end of the quote.

Have you considered this before, this language from the NCI website, as you examined scientific evidence and reached your opinions?

MR. FROST: Objection to form.

A. Well, as I mentioned, this appears to be a website for general patient information. I don't see any references

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behind the -- that talc section as you described it. I haven't had a chance to look at it in more detail. This isn't something that -- it's not a scientific article. I don't see any data included here.

BY MR. SOILEAU:

Q. Would you give any weight to this statement by the National Cancer Institute?

MR. FROST: Objection.

A. As I've said previously, with any agency with statements, I want to look at the science and the data that they are using or citing, and with this website, this isn't a study, and I don't see any citations or anything specifically that they put forward for that section other than those sentences.

BY MR. SOILEAU:

Q. Did you, in your report, actually cite governmental and agency summaries?

A. I'd have to look specifically, but I -- as I've -- you know, we stated before, I cite IARC and I cite other

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government agencies as well.

Q. Look at the table of contents, II, and see what 7.3.3 is. I'm sorry, did you see?

A. Yes.

Q. It's a review of governmental and agency summaries, isn't it?

A. Yes, that's what the title is.

Q. Okay. Let me show you a new exhibit. I'm sorry, I've been annoyed at the little ding that I'm hearing, and I didn't know where it was coming from and now I know, it's coming from my pocket. I apologize to everyone. I was listening to some music at the break, so I turned the sound back on.

(Whereupon, Deposition Exhibit Tuttle-21, Health Canada Draft Screening Assessment, was marked for identification.)

BY MR. SOILEAU:

Q. I've marked an exhibit as Tuttle Exhibit 21, and I'm going to ask you, Doctor, if you recognize this as the draft assessment from Health Canada?

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A. Yes.
Q. Now, this is something that appears in your reference materials, isn't it?
A. Yes, I believe it does.
Q. And I think you told me that you added Taher, the Taher paper, to your supplemental materials after you saw some discussion of both Health Canada and Taher in some of the depositions, I assume; is that right?
A. In some -- yes.
Q. Somewhere?
A. Either in depositions or the reports. I don't recall specifically.
Q. Okay. But regardless, you've now looked at both Taher and Health Canada, and you had included Health Canada in the reference materials for your original report?
A. That's correct.
Q. Okay. Let's go to pages 19, 20 and 21, and I'm really starting on the bottom of 19. You see the sentence that says: The most recent meta-analysis, Taher, et al.

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2018?
A. Yes, I see that.
Q. That's the Taher paper that you added in your supplemental materials?
A. I believe so, yes.
Q. And it goes on to say, in part, employed the Hill criteria, Hill 1965, and of course, that's the Sir Arthur Bradford Hill paper that we've discussed?
A. Again, without flipping to the references, I believe so.
Q. Okay. Look at the next paragraph, 19. You see Strength as a topic?
A. Yes.
Q. And then on page 20 you see the topics of Consistency, Specificity, Temporality and Biological Gradient?
A. Yes.
Q. Do you recognize those topics from the Hill paper?
A. Yes. Those are all some of the -- a portion of the nine viewpoints put forth by the Hill criteria.
Q. Very well.

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Let's turn to page 21 now, if we could. And you see a paragraph near the top of page 21 that begins with the words Biological Plausibility?
A. Yes.
Q. And that's what we're talking about. I mean, that's -- we sort of launched into a long discussion before our most recent break, but we were talking about plausibility or, as you say in your report at page 5, biological plausibility under the Hill paper and viewpoints, fair?
A. Yes.
Q. Okay. So you have considered what Health Canada has said about biological plausibility and specifically migration, correct?
MR. FROST: Objection.
A. I would -- again, I would need to look at my report. If -- I certainly am familiar with the Health Canada document, but again, I don't know. I'm sorry, I don't know that I understand what you're referring to as far as how I used the Health Canada document.

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BY MR. SOILEAU:
Q. That's fine. That's a fault with my questioning then, and let me fix it.
If something is on the reference list that is attached to your report, Doctor, does it mean that you reviewed it, or is it possible that it's on the list but you never looked at it?
MR. FROST: Objection.
A. So everything that is cited in my report I have reviewed or read. Now, there's a large number of articles on there, so, you know, it may have been some time since they've been read or anything like that.
BY MR. SOILEAU:
Q. Okay. And I -- to be fair, I particularly didn't ask how much time or how in depth you read each one, but I did want to note that you did at least consider to some extent each of the items included in the reference materials; is that fair? You did?
A. Yes, as I said, if they're cited in my report, I've referred to them, I

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have at least read them. As I said, it may have been some time previously. It may have been a brief review versus an in-depth, but I have read all the articles that I cite in my report.

Q. Okay. Let's look at what Health Canada said on page 21 under Biological plausibility. Particles of talc are hypothesized to migrate into the pelvis and ovarian tissue, causing irritation and inflammation. The presence of talc in the ovaries has been documented (Heller, H-E-L-L-E-R, et al, 1996b). This evidence of retrograde transport supports the biological plausibility of the association between perineal talc application and ovarian exposure.

Did I read that portion of the paragraph correctly?

A. Yes, but you stopped mid-sentence there at the end.

Q. Let me keep going then. We'll go all the way to the end of the paragraph.

However, the specific

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mechanism(s) and cascade of molecular events by which talc may cause ovarian cancer have not been identified (Taher et al, 2018).

Now, we've read the entire section on biological plausibility, correct?

A. Well, we've read that entire paragraph where it specifically addresses biological plausibility in that specific --

Q. Fair. Right. I didn't mean to say that there's no mention of plausibility elsewhere in the paper. I just meant that we've gone through the Hill viewpoints. We got to biological plausibility, and I just read the section on biological plausibility.

The next thing in Health Canada on page 21 is Coherence, another viewpoint, right?

A. Yes. As I said, on that particular page. I don't recall if it's discussed in more detail elsewhere in the report, but in this particular section, you've read the entirety of the plausibility paragraph.

Q. Thank you.

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Did you give any weight to this statement by Health Canada?

MR. FROST: Objection.

A. Well, again, as I've stated before, in any of the government or regulatory agencies, when reviewing their documents and summaries, I try to look at the data that either supports or doesn't support their statements or the references they cite.

In this particular paragraph, they cite the Heller study, which I believe I cite in my report.

Q. You do.

A. And as well as the Taher meta-analysis, and to be clear, the Heller study, I would need to have it in front of me, but it states here, only looks at the presence of talc in the ovaries. It does not document or assess external application and migration, and the Taher study, as I understand, is a meta-analysis of the epidemiological studies, so again, does not assess the migration theory.

Q. Doctor, in your opinion, has

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talc been found in ovarian tissue?

A. Well, I --

MR. FROST: Objection.

THE WITNESS: Sorry.

A. Again, as I stated, there have been studies that have looked at talc in ovarian tissue -- we'd have to look at those individual studies, you know, but -- has seen it, but again, has not been looking at the external perineal application and migration.

BY MR. SOILEAU:

Q. Yes, Doctor. This is a different question, though. I'm not asking you about the migration at this moment. I'm asking you specifically, based on your review of the scientific evidence and literature, has talc been found in the ovaries of women?

MR. FROST: Objection.

A. Again, I'd have to look at the scientific evidence from a different angle as you said because as I said before, I've been looking at the evidence for migration theory from external application.

I certainly am aware, as you

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say, like in the Heller study and in other studies, they have been looking for and through their various methods have surmised that they have identified talc in the ovaries, but that was not my specific objective. It was looking at the migration theory rather than just the presence of talc in the ovaries, because we kind of discussed that earlier, that the presence of talc in the ovaries is not sufficient to indicate, or of any chemical on an organ, to indicate that an adverse health effect will occur.

BY MR. SOILEAU:

Q. Do you believe that talc has been found in the ovaries of women who have had their ovarian tissue examined based on your review of the scientific evidence?

MR. FROST: Objection.

BY MR. SOILEAU:

Q. I'm not asking about migration. I'm not asking about carcinogenicity right now. I'm asking you, based on your review of the scientific evidence, has talc, talc particles as you say in your report, been

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found in -- embedded in ovarian tissue?

MR. FROST: Objection.

A. Well, again, in this particular report and in this -- because I did not do an assessment regarding just the presence of talc in the ovarian tissue. We can refer to my report where I discuss it in more detail regarding those things.

I think others involved in this litigation get more in detail into that. I merely looked at it through the lens of the Hill criteria as far as the migration theory that perineal application can migrate to the ovaries.

Q. You say this particular report. You're talking about the report that you authored and signed in this litigation?

A. Yes, I'm referring to -- to the objectives of my scientific -- the research that I put in my report.

Q. You did look at Heller, didn't you?

MR. FROST: Objection.

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BY MR. SOILEAU:

Q. That is the Heller paper cited at page 21?

A. Yes, I cited it in my report.

Q. If I asked you to assume that talc particles have been identified in ovarian tissue, what explanation would you offer to help us understand how it got there?

MR. FROST: Objection, outside of the scope of this witness' opinions being offered in this case.

BY MR. SOILEAU:

Q. Well, do you think that's outside the scope of your expertise or opinions, talking about how it gets there?

A. Well, as I said, I was asked to look at the potential causal association between perineal talcum powder exposure and ovarian cancer. A small subset of that in Hill criteria is biological plausibility.

So I assessed the scientific evidence on whether the mechanism of external application and migration to the ovaries has been -- if there's been any evidence in the

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scientific literature to support that external migration, which I state my opinion has not.

But regarding just the -- you know, alternative -- as you said, the presence of talc in the ovaries and how it gets there is -- I don't believe I -- beyond the realm of talcum powder and external migration, I'm not sure that I assess any of that in my report, and I didn't look at alternative talc exposures or other potential sources of talc or things of that nature.

Q. Let me understand, Doctor. You looked to see if talcum powder applied to the perineum could migrate through the vaginal tract to the ovaries, fair?

A. So what I looked at is we -- you have the hypothesis --

Q. Yes.

A. -- that talcum powder applied to the perineum can enter the vagina and pass through the female reproductive tract to the ovaries.

What I have done under the

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biological plausibility is look to see if there's any data to support that hypothesis that the external application of talcum powder can enter the vagina and migrate to the ovaries.
Q. Well, wouldn't evidence of talc in the ovaries be relevant to the discussion and investigation of whether talcum powder can migrate through the vagina to the ovaries?
MR. FROST: Objection.
A. Well, it would depend. You know, as we talked about with some of the studies that you placed in front of me earlier, we're talking about, you know, vaginal insertion or, you know, other scenarios that are different from just perineal application.
So it would depend on the nature of the study and the nature of the assessment to determine whether it would be applicable.
BY MR. SOILEAU:
Q. But you haven't looked.

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MR. FROST: Objection.
BY MR. SOILEAU:
Q. You haven't looked at the scientific literature and evidence to form an opinion on whether talc is found in the ovaries of women; is that correct?
MR. FROST: Objection.
A. Again, I looked at the scientific evidence to see whether there have been any scientific evidence to support that talcum powder applied externally to the perineum can enter the vagina and transport through the female reproductive tract to the ovaries.
I think that there are others involved in this litigation that get more into some of the questions you're asking, and so I would probably -- have to refer to their work and then again look at what they cite to do that type of assessment.
BY MR. SOILEAU:
Q. You would have to rely on what they say?
A. No, I said I would refer to

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their -- what they did. I know they went more -- as I said, I believe there are others who have gone into more detail, and so I would refer to their work. Because as I said, I just looked at the scientific evidence for an external application migration hypothesis.
Q. We talked about Johnson & Johnson documents. Were you provided with any Imerys documents?
A. I believe there are some Imerys documents put in my -- on my materials relied and reviewed upon. Anything I was provided is listed there on my report.
Q. Okay. Those are the materials regarding the analysis of the content of the talcum powder products and the makeup of fragrances?
A. Yes, that's correct. I believe so.
Q. And by that, I simply mean the appendices on those issues, and those, you're telling me, might include some reference to Imerys documents in part.

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Do I have it right?
A. I'm sorry.
Q. You want me to try again?
A. Please.
Q. It's possible that you have some Imerys documents referenced in some of the appendices to your report, fair?
A. Yes, and...
Q. Let me show you a document that I've marked as Tuttle Exhibit 22. It's Bates numbered as JNJ_000704 through 709 consecutively, and ask if you've seen this document before.
(Whereupon, Deposition Exhibit Tuttle-22, 2004 Fax Transmission of Sj?sten Publication, was marked for identification.)
A. No, I'm not familiar with this document.
BY MR. SOILEAU:
Q. Okay. Let's look at on the first page, you see what I've highlighted on the screen just to give us a sense of where I'm at? I'm reading here. I tell you what.

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I'll read the entire note.
I came across this paper this morning published in the April 2004 journal, Human Reproduction, an official journal of the European Society for Human Reproduction and Embryology. It offers some compelling evidence in support of the, quote, migration, closed quote, hypothesis. Combine this, open quotes, evidence, closed quotes, with the theory that talc deposition on the ovarian epithelium initiates epithelium inflammation - which leads to epithelium carcinogenesis - and you have a potential formula for NTP classifying talc as a causative agent in ovarian cancer.
Did I read that correctly, Doctor?
A. Yes, you read that correctly, but this appears to be a personal communication with some personal conclusions and opinions. I don't know, you know, the basis for all of those statements or conclusions. As you say, he attaches the -- and I'm not going to be able to pronounce the

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last name, but the S-J- --
Q. Sj?sten?
A. Yes, that we discussed earlier.
Q. Right. So this is the paper we discussed earlier, which is now Tuttle Exhibit 18, that is, the Tuttle Exhibit 18 is apparently attached to this note from Richard J. Zazenski, Z-A-Z-E-N-S-K-I, director of product safety, to Bill Ashton, fair?
MR. FROST: Objection.
A. That's -- it appears that, yes, the article is attached to this communication.
BY MR. SOILEAU:
Q. And this Sj?sten paper is from 2004?
A. Again, this was not something I cited in my report, so I'm not --
Q. I understand that. Go ahead. I'm sorry.
A. -- very familiar with it. In the communiqué? it says published in April 2004. I --
Q. Right. And you have the paper.

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A. I've not seen the public -- the publication date on the papers attached right here on the top.
Q. Right. But I gave you the paper as Tuttle Exhibit 18, if it helps. It will be much easier to read, I think, than the small print on what is now Tuttle Exhibit 22.
This is a pretty important statement if it's accurate, isn't it?
MR. FROST: Objection.
BY MR. SOILEAU:
Q. Well, if -- I mean, doesn't this statement on Tuttle Exhibit 22 cause you to want to pause and revisit the question of migration?
MR. FROST: Objection.
A. Again, as I said earlier, this is a personal communiqué?. There are several statements and "mays" and, you know, "potential" and, you know, things, and this is just one article that they cite. I don't know the context of this conversation. I don't know Mr. Zazenski, I don't know what

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he's basing these statements on.
And again, it's a personal communication. It's not a scientific article or a scientific journal beyond the one article that they -- he attaches in this communication.
BY MR. SOILEAU:
Q. Right. I understand what you're saying, Doctor, but doesn't this document at least cause you to wonder, to want to pause and do more investigation, to find out who Mr. Zazenski is, for example?
MR. FROST: Objection.
A. Again, it's an internal communication. It's not a scientific document. You know, it's not something that I would refer to in doing a review of the scientific literature.
BY MR. SOILEAU:
Q. If he was available, would you not want to ask him why he finds this paper to be compelling evidence in support of migration, the migration hypothesis?
MR. FROST: Objection.

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A. Again, he makes several statements, and -- without knowing the references or the basis for his opinions, but again, it's a -- his opinions. It's a personal communication. It's not something I would use in doing a scientific assessment.
BY MR. SOILEAU:
Q. Do you know who NTP is? It's referenced at the second-to-last line of this note to Mr. Ashton.
A. I know from my experience who NTP would be, but I don't know if it's the same organization, you know, as the -- Mr. Zazenski is referring to.
Q. Who do you know it to be?
A. I know NTP to be the National Toxicology Program.
Q. It would make sense, wouldn't it, that that is who he is referring to? You have a potential formula for NTP classifying talc as a causative agent in ovarian cancer.
MR. FROST: Objection.
A. Again, I know who -- that's who I would think NTP meant, but I'm not aware.

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There may be other organizations that have that same acronym. I don't know.
BY MR. SOILEAU:
Q. Let me show you another statement in the Hill paper. He quotes Holmes and Watson and says, quote, "When you have eliminated the impossible, whatever remains, however improbable, must be the truth."
Do you see that?
A. I'm sorry, can you refer me to the --
Q. The exhibit number?
A. Yes, please.
Q. I'll be glad to. It is Tuttle Exhibit 7.
A. Thank you.
Q. The paragraph says, in full, at the top of page 10 on the right column: In short, the association we observe may be one new to science or medicine, and we must not dismiss it too lightheartedly as just too odd. As Sherlock Holmes advised Dr. Watson, quote, "When you have eliminated the

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impossible, whatever remains, however improbable, must be the truth."
A. I'm sorry, is there a question?
Q. Do you think that's good guidance?
MR. FROST: Objection.
A. Well, again, you're taking the summary statement of what he discusses in plausibility where, you know, he says that it depends on the biological knowledge of the day, and he quotes several things, and that is his summary statement regarding, you know, looking at plausibility.
BY MR. SOILEAU:
Q. All right. Let's look at one more. I'll mark this as Tuttle Exhibit 23.
(Whereupon, Deposition Exhibit Tuttle-23, 4/1/14 FDA Letter, was marked for identification.)
THE WITNESS: And if we may after this one, can we take a short break?
MR. SOILEAU: Absolutely. I thought it was about time myself.

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BY MR. SOILEAU:
Q. Here is Exhibit 23. It is a letter from the Food and Drug Administration. I have a date of April 1, 2014. It's from the Department of Health and Human Services. Certainly you recognize the Food and Drug Administration, correct?
A. Yes.
Q. Just as an entity?
A. Yes, I'm familiar with the Food and Drug Administration or FDA.
Q. Okay. And do you recognize that the Food and Drug Administration has some jurisdiction or authority in connection with cosmetic products?
MR. FROST: Objection.
BY MR. SOILEAU:
Q. If you know. You may not know.
A. I -- you know, I know that the FDA has done several things in regards to cosmetic products regarding a specifical -- specific authority or regulations. I don't -- we'd have to be more specific. That's a very kind of general, overarching...

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Q. Okay. Do you know whether Shower to Shower and Johnson's baby powder fall under the authority of the Food and Drug Administration?
MR. FROST: Objection.
A. Specifically, I don't know. I didn't look at that.
BY MR. SOILEAU:
Q. Okay. Let's look at page 5 of 7. And I'm going to direct you to this paragraph, which is the third full paragraph. It begins with: While there exists no direct proof of talc in ovarian carcinogenesis.
By the way, we looked at something earlier. Isn't it true that in cancer, the mechanism, the exact mechanism is often not known --
MR. FROST: Objection.
BY MR. SOILEAU:
Q. -- but the association, the relationship, the causal relationship can be established?
A. Well, that's a very broad statement. Previously when we were talking

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about the Hill criteria, as I said before, you don't take any one particular criteria in a vacuum. You look at all nine viewpoints, the context and the body as itself.
And in areas where the strength and consistency in association are very strong, the evidence regarding a mechanism may be not as strong --
Q. Exactly.
A. -- and you have a very strong evidence forward. If you don't have a strong association or strong consistency or, you know, strong strength of the association, then the burden on the other criteria becomes much greater as far as looking at the nine criteria as a whole.
Q. So, for example, in smoking we may not know the exact mechanism of the carcinogenicity, but given the strength of the association, there is no doubt that there is a proper conclusion that smoking causes lung cancer, as an example, fair?
A. I haven't researched the mechanisms regarding cigarette smoking and

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lung cancer.
Q. Never mind.
It says, going on in this paragraph: The potential for particles to migrate from the perineum and vagina to the peritoneal cavity is indisputable. It is therefore plausible that perineal talc (and other particulate) that reaches the endometrial cavity, fallopian tubes, ovaries and peritoneum, may elicit a foreign body type reaction and inflammatory response that in some exposed women may progress to epithelial cancers.
Do you see that?
A. Yes, but you ended before the last sentence of that paragraph.
Q. Sure. It goes on to say: However, there has been no conclusive evidence to support causality.
All right, now we've read the entire paragraph and a couple of stops and the document will speak for itself.
But it does say the potential for particulates to migrate from the perineum

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to the peritoneal cavity is indisputable, doesn't it?
MR. FROST: Objection.
A. Again, that's what it states here, but it doesn't provide any basis for that statement.
BY MR. SOILEAU:
Q. And certainly you disagree with that?
A. As I said, it's not for me to agree or disagree. There's no basis for the statement as put forward here for me to look at the data that they used for this particular statement, and as I've said before, the scientific evidence does not support the external application migration theory for talcum powder.
Q. Well, Doctor, you've been put forward as an expert witness in this case, and you have told me that migration is something that you have examined and will comment on, and I'm simply asking: Is the position that you have adopted in this litigation on behalf of J&J in conflict with

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what the FDA says here?
MR. FROST: Objection.
A. Well, as I said, this is one paragraph taken out of the entire document, and so without looking at the context and there's no references cited for me to look at their basis for this statement.
As I've said before, the scientific evidence does not support the migration theory that external application of talc can migrate to the ovaries.
BY MR. SOILEAU:
Q. Can you say whether this paragraph conflicts with the opinions you have offered in this case and that we are discussing today?
MR. FROST: Objection.
A. Again, without looking at the -- you know, the document in its entirety, we're taking it out of context, I certainly agree with the statement there's been no evidence to support causality at the end of the paragraph and that there's no direct proof of talc and ovarian

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carcinogenesis.
BY MR. SOILEAU:
Q. So you agree -- I'm sorry.
A. These -- well, these -- again, the scientific basis for this is not put in here. There's no references. We would need to look at all the data beyond -- you know, this is just a letter regarding, if I remember correctly, a petition, where they're addressing a petition, rather than a document that is doing any -- any testing.
I think they summarize some information in here, but I'm not seeing, you know, references or you know, a set of scientific data to refer to or review as far as the scientific evidence.
Q. But you're telling me at the same time, you certainly agree with the statement that there's been no evidence to support causality?
A. Well, as I --
Q. Is that right?
A. As I said before, I have not found any scientific evidence that -- or,

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excuse me, the scientific evidence does not support the external application of -- migration theory that the external application can migrate through the female reproductive tract and reach the ovaries.
But as I have -- I think I've also said before that the scientific evidence does not support a causal association between perineal talcum powder exposure and ovarian carcinogenesis.
Q. Whether you agree or not, in this paragraph, the FDA is telling us that it is biologically plausible, that is, migration of talcum powder through the vagina to the ovaries is biologically plausible. FDA is telling us that here, whether you agree or not, isn't it?
MR. FROST: Objection.
A. Again, we've quoted the paragraph out of this letter --
BY MR. SOILEAU:
Q. Right.
A. -- again, addressing petitions. There's -- again, we haven't looked at the

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scientific data behind the statements or behind any -- any of the other things that we've discussed.
Q. Shouldn't you have done that before today?
MR. FROST: Objection.
A. Well, as I said previously, I've assessed the body of science in my report and looked at the external application of perineal talc and the potential migration to the ovaries as well as the other nine viewpoints of the Hill criteria and the -- whether the scientific evidence is supportive of a causal association between perineal talcum powder exposure and ovarian cancer, for which the scientific evidence doesn't support that causal association.
And the scientific evidence does not support the hypothesis that the external application of talcum powder cannot migrate -- or that can migrate to the ovary after external application.
BY MR. SOILEAU:
Q. Had you seen this document

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before today?
A. Yes, I believe so.
Q. Can you at least tell me then, having reviewed this document previously and having it before you today, that the FDA, regardless of whether you agree or disagree, that the FDA is finding biological plausibility in the context of that topic that we've discussed today in this letter? They're saying it is therefore plausible?
MR. FROST: Objection.
A. Well, again, this is -- this is a letter. This is a response to a petition. I don't see that this is a research article or a -- you know --
BY MR. SOILEAU:
Q. Can you even say, though, Doctor, what the letter says? Yes, it's a letter. Yes, it's a letter though from the FDA. No, it doesn't cite literature. But I'm asking you: Does the letter say it is biologically plausible?
MR. FROST: Objection.
A. Again, you know, we have read

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the paragraph, and in the paragraph it doesn't say biologically plausible.
BY MR. SOILEAU:
Q. Okay. I mean, look at page 28 of your report. Aren't you citing in the paragraph that begins: In 2014, the FDA -- aren't you citing this very letter that I've marked as Tuttle Exhibit 23?
A. Yes, I am. It's in the subsection we were discussing earlier entitled Governmental and Agency Summaries.
Q. Right. And you quoted from this letter.
A. Yes, I do.
Q. Even though the letter doesn't include any citation or authority.
A. Well, again, as I've said before, with governmental agencies and documents, I refer to them, I look at their information, and then I think it's important to look at the scientific data.
But again, this, again, also finds that data submitted presented conclusive evidence -- or excuse me, did not

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find that the data submitted presented conclusive evidence of a causal association.
Q. What methodology is it, Doctor, that allows you to quote this letter for that position, yet ignore the statement of plausibility in the same letter by FDA?
MR. FROST: Objection.
A. So again, I was asked to opine on the overarching scientific data, the body of science, regarding the causal association between talcum powder and ovarian cancer and the scientific evidence of whether it supported the hypothesis that there's an association.
In looking at the body of evidence and looking at the Hill criteria and looking at all the criteria, that was my objective of which biological plausibility, you know, I address, again, is one portion.
BY MR. SOILEAU:
Q. Right, but in doing that, you cited --
MR. FROST: Let her finish her answer.

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MR. SOILEAU: Go ahead, if you're not finished.
A. And as I said previously and we talked about previously with the Hill criteria, you can't take one criteria in a vacuum and just examine that solely for the establishment of causation. You need to look at the entire context.
So in this particular scenario where I was looking at this letter, I do cite that their -- you know, their overarchal conclusion regarding causal association, but again, my research is in the scientific literature and the peer-reviewed literature as far as these provide context and information.
BY MR. SOILEAU:
Q. But what you have done is you have, in fact, taken one statement from the FDA letter -- you've taken a statement from that letter from the FDA, cited it in support of no evidence of a causal association, and ignored its statement about biological plausibility when you tell us later that

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there's no evidence of plausible theory.
Haven't you done the very thing -- you need me to say it over? You took one part of this FDA letter and put it in your report and relied on it, didn't you?
MR. FROST: Objection.
A. Well, again, as I stated before, when looking at governmental agency summaries and documents, you know, while I refer to them and I look at their conclusions, I look at the scientific data that is the basis for those conclusions.
And in this particular section that you're referring to with the FDA quote that I used, the government and -- governmental and agency summaries, if -- I'm jumping ahead in my report a little bit, but I was looking at the --
BY MR. SOILEAU:
Q. Right.
A. -- determinations regarding a causal association. In this particular section, I'm addressing causal association, not biological plausibility, which I indicate later in my report.
Q. Yes, Doctor.
A. Which I state that no scientific evidence supports the migration theory.
Q. I got that.
A. What is stated here is not scientific evidence. It's a sentence in a letter.
Q. Yes. But these are all apples from the same tree, the tree of this letter, and you have used one of the apples that you selected on causation and ignored an apple on plausibility as part of your methodology, haven't you?
MR. FROST: Objection.
A. No. Again, as I said, I looked at the body of science and the scientific evidence. The citing of this particular quote for the FDA article under the governmental agencies, as I said, it's about the science and the body of science.
It's not about, you know, each individual governmental agency of which, you know, I refer to several in this particular section, and I cite several in my report.
MR. SOILEAU: All right. Let's take our next break now. Thank you.
THE VIDEOGRAPHER: Going off the record at 1:56 p.m.
(Recess taken, 1:56 p.m. to 2:07 p.m.)
THE VIDEOGRAPHER: We're back on the record at 2:07 p.m.
BY MR. SOILEAU:
Q. Okay. Doctor, are you ready to proceed?
A. Yes, I am.
Q. Very well. Thank you.
Would you consider evidence of talc in the ovaries of women to be important, relevant evidence that you would want to consider in your evaluation of the causality question that you described earlier?
MR. FROST: Objection.
A. So I think we discussed that earlier in that the presence of talc in the ovaries is -- does not necessarily mean that an adverse health effect will occur. We have to look at the body of science as a whole and the scientific literature beyond -- beyond that to see if there is a causal association.
BY MR. SOILEAU:
Q. I understand that the presence of talc in the ovaries does not end the causal association analysis, but I'm asking you if it is one step, one part of the evidence that you, as a toxicologist, would want to know about and to consider in your work on this project.
A. So again, as I stated before, I wanted to assess the hypothesis on whether external perineal application of talcum powder can cause migration to the ovaries, for which the scientific evidence does not support that hypothesis.
Q. I've heard that from you, but that's not the question I'm asking you. I really need you to answer my question; and that is: If there is evidence, okay -- if there is evidence of talc in ovaries, do you simply say, well, that does not necessarily

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mean that an adverse health effect will occur, so we don't need to look at it?
MR. FROST: Objection.
BY MR. SOILEAU:
Q. Is that consistent with the methodology that you've used here?
MR. FROST: Objection.
A. No. So what I'm saying is that the question that we are asking or the question that I was asking in doing my literature -- assessing the body of science is whether the perineal application of talcum powder is causally associated with ovarian cancer.
The subpart of that, as we've been discussing, is whether the migration theory that the external application of talcum powder can migrate from the external genitals to the ovaries.
When you ask about just the presence of talc in the ovaries, that is -- you know, we need to look at the mechanism, not just the mere presence. That's what I mean when I say the presence does not mean an

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adverse health effect will occur.
We have to look at the scientific literature. We've talked a little bit about the plausibility, but also the Hill criteria, the epidemiology, the evidence, because the -- as opposed to just the mere presence.
BY MR. SOILEAU:
Q. You said that one part is migration, the issue of migration. Did I get that right?
MR. FROST: Objection.
A. I --
BY MR. SOILEAU:
Q. You said -- I'm sorry, I'm going to use your words so that we have it more fairly and correctly.
The subpart as we've been discussing is migration. That's not a full quote, but I'm using your words to structure my question, to be fair.
The subpart that we've been discussing is migration, the migration theory. I'm simply asking is the presence of

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ovarian tissue with talc, that is, the presence of talc in ovarian tissue, another subpart that you would and should consider in your analysis of these issues?
A. So again -- and I don't have my words in front of me, but as I was talking about the question we're looking at is the association between talcum powder use perineally and ovarian cancer and whether there is scientific evidence that supports a causal association.
So the presence of talc in the ovaries or evidence noting talc in the ovaries, as I said, we have to look at -- that's one part of -- but as we were talking about with biological plausibility, we have to look at the hypothesis that talc, when applied externally to the perineum, can migrate to the ovaries. And the scientific evidence does not support that hypothesis.
In regards to just the presence or absence of talcum powder on the ovaries, that's just a very, very general, you know, looking at the scientific literature, is it

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there, isn't there type of scenario as opposed to the specific question we're looking at regarding talcum powder use perineally and ovarian cancer, which is what I assessed the scientific literature for.
And as I've said previously, you know, there are other that get into the biological plausibility, the migration theory in more detail than I do. But in the context of my report and in the context of the body of science, we're specifically looking at that relationship between perineal application and ovarian cancer.
Q. Doctor, was it purposeful that you changed my question from talc in the ovarian tissue to talc on the ovary?
MR. FROST: Objection.
A. I'm sorry. That was not intentional.
BY MR. SOILEAU:
Q. Okay. Do you recognize a distinction there between talc in the ovarian tissue versus talc on the ovary?
A. Again, that was just a -- I --

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no, there was --
Q. Okay.
A. -- that was not intentional to change it in that manner.
Q. Okay. Well, in my review of the literature I've seen that distinction made, and that's why I was wondering if you were moving to a different issue. I was confused. It was a long answer.
Is evidence of talc in the ovarian tissue evidence that you would consider in your analysis of the causation question?
MR. FROST: Objection.
A. Again, I would need more information. As I was talking about, we -- in the causation question we're assessing, we're looking at perineal application of talcum powder and ovarian cancer.
So just saying if the talc is present or not, we would need more information as far as using that, the scientific evidence for that in assessing a causal relationship between talcum powder and

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ovarian cancer.
Q. So to be clear, if all I have is there is evidence of talc, talc particles in the ovarian tissue, that standing alone is not enough to move the ball for you?
MR. FROST: Objection.
A. Again, you have to have more information. You have to look at the context. You have to look at the studies that you're referencing for scientific evidence. You have to look at it in the context of the question and the scientific evidence as a whole.
BY MR. SOILEAU:
Q. Right. And what I want to understand is the boundaries around, the fence line around the scientific evidence as a whole and what it takes to get within those boundaries.
And what I hear you telling me is evidence of talc particles in ovarian tissue, just that, none of the other things you mentioned, context, so forth, that's not enough to get within that body of evidence

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for you.
Do I have it correct now?
MR. FROST: Objection.
A. Well, as I said, I need more information about the scientific evidence that you're purporting.
BY MR. SOILEAU:
Q. Right. You need more information in order for that evidence to get inside the fence, to get within the body of scientific evidence that you're considering, fair?
MR. FROST: Objection.
A. I'm saying that I would need more evidence about -- about the evidence. You've made the statement of evidence that talc present in the ovaries, but I would need to look at the studies, look at the questions that are being asked in the studies, you know, what the data they used, the dataset itself, because again, we're looking at a specific question regarding perineal application of talcum powder and ovarian cancer, not just the potential presence of

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talc particles on or in the ovaries.
BY MR. SOILEAU:
Q. Do you recognize the name A.P. Wehner?
A. No, I do not.
Q. You do not?
A. Huh-uh.
Q. Let me show you what I'll mark as Tuttle Exhibit 24.
(Whereupon, Deposition Exhibit Tuttle-24, 1994 Wehner Publication, was marked for identification.)
BY MR. SOILEAU:
Q. It bears a JNJ Bates-numbering of 14178 through 14189. It's a Wehner 1994 paper.
Do you understand generally what is meant by the words "hygienic talc use"?
A. I -- again, I know what I would think it would mean as hygienic talc use, but if somebody was using it, I would probably want to know what their definition was when they used it. I wouldn't want to assume my

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definition.
Q. What's your understanding? I mean, you're here as an expert witness for Johnson & Johnson on these talcum powder products. What's your understanding of hygienic use, hygienic talc use?
A. That would be -- again, my understanding and I would have to see how it was used in context, but for personal hygiene purposes.
Q. Does that mean application to the genital area?
MR. FROST: Objection.
A. Again, I would have to see the context in which it was being used.
BY MR. SOILEAU:
Q. Okay. And you do not know who A.P. Wehner is, as you now look at what I have marked as Tuttle Exhibit 24?
A. Again, I'm not familiar with the name off the top of my head.
Q. Okay. You see that this is a 1994 paper from Wehner entitled Biological effects of cosmetic talc?

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A. Yes, I see that.
Q. Do you know if A.P. Wehner has any association with Johnson & Johnson or any connection to Johnson & Johnson?
MR. FROST: Objection.
A. I don't know.
BY MR. SOILEAU:
Q. Do you know if you cited any papers by A.P. Wehner in your reference materials?
A. I'd need to go to the reference section of my report and look.
Q. Take a look and see if you see about four there.
A. Thank you.
Q. Would that surprise you? W-E-H-N-E-R.
A. Thank you. I -- yes, I do cite one two, three, four A.P. Wehner reports in my reference materials.
Q. Do you know how you came to have those four reports?
A. Again, I know how I came to find all of the reports that I cite, and

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those are through the PubMed, Google Scholar, you know, scientific literature searches that we discussed previously.
Q. Let's look at -- well, first I should be clear.
Is this paper, the Biological effects of cosmetic talc, from 1994, the same paper I now have marked as Tuttle Exhibit 24, one of the four papers that you cite in your reference materials attached to your report?
A. Yes, it is.
Q. Okay. And doesn't Wehner address the presence of large numbers of talc particles in normal and diseased ovarian tissue in this paper, or do you know?
A. Again, I would need to refamiliarize myself with this report. It does appear to be a review article. If you can refer me to that specific area you're discussing, I can flip to it.
Q. Sure. Let's look at the discussion which begins on page 1181 of the paper. You see the heading Hygienic talc use and ovarian cancer?

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A. Yes.
Q. If you could follow along: The presence of large numbers of talc particles in normal and diseased ovarian tissue seems indisputable. Although it is difficult to imagine how inanimate particles without locomotion of their own can breach the formidable cervical barrier and migrate upstream -- upstream is in quotes -- against the ciliary beat of the fallopian epithelium to the ovaries.
I read that sentence correctly?
A. Yes, you read that sentence correctly. Again, it's one sentence out of this whole paragraph.
Q. But now I'm using a paper that you cited and relied upon. This paper is not new to you?
A. Correct. Again, I cite a lot of different articles. As I mentioned before, I read them all, but it's a large number of articles. I unfortunately do not have a photographic memory where I can remember everything written verbatim.

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Q. Do you consider this important scientific evidence?
A. So --
MR. FROST: Objection.
THE WITNESS: Sorry.
BY MR. SOILEAU:
Q. Well, you've talked today a number of times about the body of scientific evidence, so perhaps I could use your words and frame my question this way.
Is this statement in the Wehner article part of the body of evidence, scientific evidence, that you have considered in forming your opinions?
A. Well, again, I read and cited this article. It is a review article, so it does not do any research in and of its own. There are no -- there's no datasets included in this article. There's no methodology or experiments that were performed by Dr. Wehner in this article.
This is a review article that is reviewing the body of science. And in this particular sentence, again, there's no

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citations for that particular sentence. You know, and the paragraph goes on in discussing epidemiological evidence and other components, but...
Q. So did you rely on this article in forming your opinions, or not?
A. Again, I reviewed and cited this article, but there's no data in here. There are references to cite. There's -- but there's no experiments that were performed for examination or to determine the basis for that statement. And again, they don't cite any references specifically here for me to refer to.
Q. Why did you list this article in your references?
MR. FROST: Objection.
A. Because again, it is a review article that is titled the Biological effects of cosmetic talc. I believe I probably cite several review articles in the references of my report.
BY MR. SOILEAU:
Q. Yes.

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A. And they're useful for, you know, looking at a review of the literature, finding articles and, you know, scientific publications that have done experiments and provided data points.
Q. So is the Wehner paper that is Tuttle Exhibit 24 in or out for the body of evidence that you considered, the body of scientific evidence?
MR. FROST: Objection.
A. Well, again, as I said, it's a reference that I referred to and that I cite in my report, and looking at all of the references that we -- I cite in my report and the scientific data, it's looking at the body as a whole of the scientific data and what supports those conclusions.
As I said, this is a review article so they're reviewing scientific evidence and coming up with their opinions and hypotheses, again, looking at this one sentence out of context. There's no references cited here. I know that later in this same paragraph it discusses that the

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epidemiological evidence linking hygienic talc use with an increased risk of ovarian cancer generally is weak, is sometimes inconsistent, discusses confounding variables.
And again, these are summary statements taken out of the discussion, but there's no references taken, and we can't just look at that one statement without looking at the context of the entire document.
And as I said for you, if you go over to the next page and look at 1182 under Conclusion, it looks -- as they say, there's no conclusive evidence in the literature reviewed to indicate that cosmetic talc, when used as intended, presents a health hazard.
BY MR. SOILEAU:
Q. I think that answer was over 30 lines, Doctor, and really, I did not hear an answer.
Is this paper part of the body of evidence that you considered part of the

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body of scientific evidence, using your words throughout your testimony today?
MR. FROST: Objection.
A. Well, again as I said --
BY MR. SOILEAU:
Q. If you could tell me it is, it isn't or for some reason you can't tell me, and then give me that long answer if you need to again. But I need to know: Is it -- I'm looking at that fence line. I need to know if it's inside the fence or outside the fence. Can you tell me?
MR. FROST: Objection.
A. Well, I'll try to simplify my answer, that it's --
BY MR. SOILEAU:
Q. It's real simple. Yes, no, in, out. Go ahead.
MR. FROST: Objection.
A. In regards to the fence, as I said, it's a review article that I cite in my report. I certainly read it, reviewed it, as I said I cited probably several review articles in the report.

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So as far as being in the scientific literature, yes, but this does not produce any experiments or new datasets. It merely is also reviewing the scientific literature.
Q. You think Wehner knew what he was talking about?
MR. FROST: Objection.
A. As I don't know Dr. Wehner...
BY MR. SOILEAU:
Q. Doesn't it kind of remind you of the Hill statement when he says: It's difficult to imagine how inanimate particles without locomotion of their own can breach the formidable cervical barrier, yet it's there?
Do you recall the statement by Hill, the Holmes/Watson statement, when you rule out the impossible, the improbable remains?
MR. FROST: Objection. Is that a question?
BY MR. SOILEAU:
Q. Sure. I mean, isn't this what

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science is all about? We've got -- we've got talcum powder applied to the genital area, and we've got, according to Wehner, indisputable evidence of large numbers of talc particles in normal and diseased ovarian tissue. As a scientist, we need to kind of scratch our head and figure out how it's getting there, don't we?
MR. FROST: Objection.
A. Well, as I said before, when we were talking about just the presence of talc particles, we need more information, you know, we need more information on the studies that they're examining.
When we're looking at the question of perineal application of talcum powder, there is no evidence to support that the migration occurs externally from the body into the female reproductive tract to the ovaries.
BY MR. SOILEAU:
Q. No evidence.
A. And as I've said, you know, previously, there are others who get into

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much more detail regarding the scientific literature regarding that hypothetic mechanism and the science regarding it.
But here again, we're talking about just the presence with no additional context regarding where that presence is found, what the studies are, what the context is.
Q. Let's look at Exhibit 25. Do you know who John Hopkins is, Dr. John Hopkins?
(Whereupon, Deposition Exhibit Tuttle-25, 3/17/97 Wehner Letter, was marked for identification.)
A. I don't know Dr. John Hopkins personally, but yes I know who Dr. John Hopkins is.
BY MR. SOILEAU:
Q. Okay. Have you seen this letter that I've marked as Tuttle Exhibit 25 before?
A. No, I have not.
Q. Do you know who CTFA is?
A. No, I'm not familiar with that

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acronym.
Q. Okay. That hasn't come up at any point during your work on this project?
A. Not -- as I said, it doesn't -- if it has, I -- yeah, I'm not super familiar with it, so I don't know what the acronym stands for. I apologize.
Q. That's okay.
You see at the top we have sort of a header for Alfred P. Wehner, Diplomate, Academy of Toxicological Science?
You see that?
A. Yes, I see that.
Q. Do you know what that is, Diplomate, Academy of Toxicological Science?
A. Judging from the context here, it would seem he has a diploma from the Academy of Toxicological Sciences, but I'm -- I'm not familiar specifically what that means.
Q. Okay. Let's look at the beginning of this letter. He says: A postscriptum to my comment faxed to you on March 16th. The date of this letter is

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March 17, 1997.
Quote, The CTFA response statement of March 10 states in its last sentence of its last full paragraph: "The determination of this workshop was that the scientific evidence did not demonstrate any real association between talc use in consumer products and ovarian tumors."
I read that correctly, didn't I, Doctor?
MR. FROST: Objection.
BY MR. SOILEAU:
Q. I know I stopped without reading the whole letter, but I read that part correctly?
A. Yes, I believe so, yes.
Q. And that statement in quotes, the determination of this workshop statement, that's consistent with what you're telling us today in your opinions and your testimony in this case?
MR. FROST: Objection.
A. Again, you know, this is in quotations. It's citing something that I'm

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unfamiliar with.
BY MR. SOILEAU:
Q. Right.
A. This is a postscript to apparently some previous communications. I can't really speak to this without knowing more information.
Q. I'm simply asking you if this statement is consistent with your position, your opinions in this litigation, that is, that the scientific evidence did not demonstrate any real association between talc use in consumer products and ovarian tumors.
Isn't that what you're telling us today?
MR. FROST: Objection.
A. Well, as I've said previously, it's not my opinion. The scientific evidence does not establish a causal association between talcum powder exposure perineally and ovarian cancer.
BY MR. SOILEAU:
Q. So you have not formed an independent expert opinion that the evidence

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does or does not support a causal relationship?
MR. FROST: Objection.
A. Again, as I said, it's not my opinion. The scientific evidence is the scientific evidence, and it does not establish a causal association.
BY MR. SOILEAU:
Q. So are you telling me that you have, through your work in this case, simply gathered scientific information and that the scientific information, the body of scientific evidence that you have gathered, does not support a causal relationship, period?
MR. FROST: Objection.
A. As I said before, I have assessed scientific literature and looked at the scientific evidence, and through the context of the methodologies that we've previously discussed and that are listed in my report, the scientific evidence does not support a causal association between perineal talcum powder exposure and ovarian cancer.

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BY MR. SOILEAU:
Q. I'm not a scientist, but when I hear someone say I've assessed the scientific literature, looked at the evidence and used methodologies, it sounds like they're forming an opinion.
MR. FROST: Objection.
BY MR. SOILEAU:
Q. Did you reach an independent opinion in this case or not?
MR. FROST: Objection.
A. Well, again, as I said, the science doesn't support a causal association.
BY MR. SOILEAU:
Q. It sounds like you're telling me the science is what the science is and there's no room for opinion.
MR. FROST: Objection.
BY MR. SOILEAU:
Q. Is that -- do I have it right now?
A. Well, again, as I said -- yes, the science is the science. The science does not change when you're looking at the body of

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science, and when you apply the methodologies as put forward in the scientific literature, there is no causal association.
BY MR. SOILEAU:
Q. So, therefore, it does not require an opinion on your part, simply a matter of gathering, assessing scientific evidence and applying the methodology to arrive at the answer.
Do I have it correct now?
MR. FROST: Objection.
A. Again, as I said, it's not about my opinion. It is about the application of the scientific methodologies as put forward in the scientific literature and the body of science, and the body of science does not support a causal association.
BY MR. SOILEAU:
Q. And you've come to that point, to that conclusion without injecting any opinion on your part, correct?
A. Again, I use my training and expertise as a toxicologist in reviewing the

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scientific literature and applying the methodology, but essentially, it is what the science shows or the data supports when you look at the method, you know, when you apply the methodology, again, as put forward again in the scientific literature.
Q. Doctor, in that process you just described for me, is there any part that is subjective opinion?
MR. FROST: Objection.
A. Again, it's an evaluation of the body of science and the application of the scientific methodology. It's what the scientific evidence says, what the scientific evidence supports or doesn't support in the case of here, and again, the scientific evidence does not support a causal association between talcum powder exposure perineally and ovarian cancer.
BY MR. SOILEAU:
Q. I've very clearly heard you tell me that over and over, but I need you to answer my question, and that is: Does this process, this evaluation of the body of

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science, the application of scientific methodology, does it include any subjective opinion that you bring to the process?
MR. FROST: Objection.
A. And again, you know, it's using scientifically validated methods, scientific data, to see what the science supports, what the scientific data points to.
As I said before, it's not about my opinions. It's about what the scientific evidence shows.
BY MR. SOILEAU:
Q. Is that a no?
MR. FROST: Objection.
A. I'm trying to be clear, and I'm sorry, I guess I'm not being. But it's not about my personal opinions. It's not about the opinions. It's about the scientific data and what the body of science shows.
BY MR. SOILEAU:
Q. So there is no part of that process that is opinion?
MR. FROST: Objection.
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BY MR. SOILEAU:
Q. Do I have it right now?
A. Again, I -- you're -- as I said, I used my training and expertise and my training in toxicology to look at the scientific evidence, but again, it's what the evidence shows.
Q. Right. And only what the evidence shows.
A. Yes. Again, it's based on the body of science and the scientific evidence and using scientifically -- methodologies that are put forward in the scientific literature.
Q. And no part of that process includes your own personal opinions, whether based on training, education, or experience or not; it's the collection of the data, assessing the data and applying the methodology, not injecting opinion.
Do I have it correct?
MR. FROST: Objection.
A. Well, again, I think I stated earlier, my training and expertise in

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toxicology are involved in how I assessed the scientific evidence.
As far as I am a toxicologist, I do toxicological research, and I research the methods and all the things that are cited in my report.
But ultimately, it's taking the scientifically validated methods, the methods established in the scientific literature, applying that to the scientific literature and the body of science and seeing what the science supports and what the scientific evidence shows.
BY MR. SOILEAU:
Q. When I make gumbo I use all the ingredients people use, but it's my opinion, my subjective opinion that determines when it's been browned enough.
You see the difference?
MR. FROST: Objection.
BY MR. SOILEAU:
Q. It's not just the ingredients.
MR. FROST: Objection.
A. Well, again -- and I think we

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discussed this also earlier, you know, as scientists, when you look at a body of science and you apply a certain methodology, there -- your conclusions or what the science shows should be the same because the body of science is not changing between individual scientists.
BY MR. SOILEAU:
Q. Do you agree with the statement in Tuttle Exhibit 25 that the scientific evidence does not demonstrate any association, any real association between talc use in consumer products and ovarian tumors?
MR. FROST: Objection.
A. Well, again, this is a quotation. There's -- I don't know the -- you know, I'm not familiar with this CTFA response statement or the data it uses for these determinations. This is a postscript to some previous comments.
Without the context of this statement, you know, regardless of what it states, I can't agree or disagree. I -- this

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is taken out of context, and again, I haven't seen this before or the CTFA response.
BY MR. SOILEAU:
Q. I hear what you're saying about the CTFA, the document, the folks referenced in here in the details. I really want to step away from that. So what I've done is I've just made a statement that is not referenced anywhere. It's -- I've marked it as Tuttle Exhibit 26.
(Whereupon, Deposition Exhibit Tuttle-26, Handwritten Statement, was marked for identification.)
BY MR. SOILEAU:
Q. And it simply says -- can you read it for me? Because that's the only copy I have.
MR. FROST: Before we get to that, I'm going to object to the use of any sort of handwritten statements where you're then asking our witness to check agree or disagree after something's written down.
MR. SOILEAU: Well, she doesn't

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have to check it, but I'm just trying to make the point of what the question is asking.
BY MR. SOILEAU:
Q. Could you read the body of the statement on that page, Tuttle Exhibit 26?
A. So the statement written is: Scientific evidence demonstrates an association between talc use in consumer products and ovarian tumors.
And then there is the options written below it to agree or disagree.
Q. And do you -- can you agree or disagree with that statement, or you do not know?
MR. FROST: Objection.
A. Well, again -- and this goes back to what we're just discussing. This is a statement that you've written on a sheet of paper. There's no context. There's no data, there's no information for me to look at or examine. You know, you've been discussing the body of science.
You know, I can't agree or

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disagree with just a statement written on a piece of paper with no additional information or context.
BY MR. SOILEAU:
Q. Do you know how many hours you have billed for your work on behalf of Johnson & Johnson in this project?
A. I don't know an exact number. I think I've billed probably around 250 to 300 hours.
Q. So you say I can't disagree -- wait a minute. Let me get your words correct.
I can't agree or disagree with just a statement written on a piece of paper with no additional information or context, but you've got hundreds of hours spent studying this very issue, right?
MR. FROST: Objection.
A. Again, I have been assessing the scientific literature regarding, you know, whether it supports a causal association between perineal talcum powder use and ovarian cancer.

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BY MR. SOILEAU:
Q. And that's what's written on Tuttle Exhibit 26, isn't it, scientific evidence demonstrates?
MR. FROST: Objection, misstates the document.
BY MR. SOILEAU:
Q. What's it say?
A. Again, it says: Scientific evidence demonstrates an association between talc use in consumer products and ovarian tumors.
Q. I've got approximately 255 hours for you if I've deciphered the hourly rate correctly. I mean, you've done a lot of work looking at this issue.
Are you prepared as we sit here today at your deposition to say that you agree or disagree with that statement on Tuttle Exhibit 26?
MR. FROST: Objection.
BY MR. SOILEAU:
Q. If you cannot, tell me that, but I need to know if you're in a position

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that allows you to agree or disagree. We can talk about why or why not in the time we have left.
MR. FROST: Objection.
A. Well, again, as I said, you know, I looked at the scientific evidence. In fact, we said this several times. In all of these articles, in all of these agency reports and anything that I cite in my review, you know, any statement that's made that's something of this nature, I would want to see the data behind that assumption because it's about the scientific data, not about the conclusions or assumptions of, you know, in any one article or any one handwritten document about the data and what the data supports or doesn't support.
BY MR. SOILEAU:
Q. Yes, Doctor, but right now the intent of my question is not about that handwritten document or the data. It's about your opinion. And I want to know your opinion. This is my chance to talk to you.
Do you have an opinion to

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offer?
MR. FROST: Objection.
A. Again, the opinions and data that I provide are summarized in my report, and regarding this handwritten statement, again, you know, I -- as it's written on this sheet of paper, I can't agree or disagree.
BY MR. SOILEAU:
Q. Okay. Now, in Tuttle Exhibit 25, the statement says that the scientific evidence did not demonstrate any real association, so that's sort of the opposite of what I wrote on Tuttle Exhibit 26, right? We can at least agree on that.
One says demonstrate, one says did not demonstrate, fair?
A. Yes, that's fair.
Q. Now, what does Alfred P. Wehner say about that --
MR. FROST: Objection.
BY MR. SOILEAU:
Q. -- in Tuttle Exhibit 25?
A. I'm sorry, can you --

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Q. Help you?
A. What do you mean by what does he say by that?
Q. Well, doesn't Alfred P. Wehner say this statement, the statement about the determination of the workshop was that scientific evidence did not demonstrate any real association. He says this statement is technically and factually incorrect, doesn't he?
A. Yes, that's what's stated, and he goes on to specify later in the postscript, he says: In summary, yes, there are those studies showing a weak association, but their biological significance is questionable for several reasons as repeatedly outlined. The data are inconsistent and therefore inconclusive. None of the authors claims to have demonstrated causality.
And again, it continues on with the rest of his letter.
Q. Let's look at one or two more before we move on. This is marked as Tuttle

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Exhibit 27.
(Whereupon, Deposition Exhibit Tuttle-27, 9/17/97 Wehner Letter, was marked for identification.)
THE WITNESS: If I may, I think we're again getting close to time that I will need to take an extended break.
MR. SOILEAU: Do we have time to take a quick look at Exhibit 27 or not?
THE WITNESS: What time did we start?
MR. FROST: I don't actually remember.
THE VIDEOGRAPHER: We've been going 38 minutes.
THE WITNESS: 38? Okay. Yeah, we can go a few more minutes.
MR. SOILEAU: Okay. Thank you.
BY MR. SOILEAU:
Q. Have you seen this before, the document marked as Tuttle Exhibit 27?
A. No, I have not.
Q. Let me sort of guide you to a

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place. You see he begins with an old German saying, and then there's a paragraph. Down in this paragraph it says: Several investigators have independently reported talc particles in ovarian tissue.
It goes on to say: Simply citing the Battelle study and stating that it demonstrated that talc does not translate -- open paren, sic, exclamation, close paren -- through the cervix to the uterine cavity and beyond does not address the problem, does not refute these findings, and therefore does not serve CTFA's best interest.
Do you see that?
A. Yes, I see that. Again, we're looking at what appears to be some personal correspondence.
Q. Okay. You see it's addressed to someone who is, at least according to this letter, in the position of manager preclinical toxicology with J&J Consumer Products?
A. Yes, I see that.
Q. All right. And the letter is

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dated September 17, 1997?
A. Yes, it is.
Q. And then if you look on page 2 at the first -- well, the second paragraph, we see a similar statement to what we talked about in the last exhibit from Wehner. It says, starting: The response statement dated November 17, 1994 is just as bad. The second sentence in the third paragraph reads: "The workshop concluded that, although some of these studies suggested a weak association might exist, when taken together, the results of the studies are insufficient to demonstrate any real association."
That's a quote.
And then he says: This statement is also inaccurate, to phrase it euphemistically.
Again, you've not seen this, you don't know who he is?
A. Correct. I have not seen this before. I'm not familiar with the CTFA and statement that they're referring to or, you know...

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Q. Does this raise a red flag in your assessment of the body of scientific evidence?
MR. FROST: Objection.
A. Again, this is -- this is a personal correspondence that's looking at response statements of a working group. There's no data here. There's no reference. I mean, it -- as you said, it referenced a Battelle study previously, but you're jumping, taking these out of context.
I -- I don't know what these letters are addressing or what the CTFA is addressing.
BY MR. SOILEAU:
Q. Okay. How about Battelle? Do you recognize Battelle at all?
A. Again, the name sounds familiar. I'm not again able to recall anything specific.
MR. SOILEAU: Okay. We can stop there and you can take the next break.
THE VIDEOGRAPHER: Going off the record at 2:49 p.m.
(Recess taken, 2:49 p.m. to 3:35 p.m.)
THE VIDEOGRAPHER: We're back on the record at 3:35 p.m.
BY MR. SOILEAU:
Q. Doctor, are you ready to proceed?
A. Yes, I am.
Q. Very well. Thank you.
Let me show you what I will mark as Appendix C -- I'm sorry, that's not correct.
I will mark it as Tuttle Exhibit 28. It is Appendix C to your report. I do not have a binder clip on this, so I'm going to give you the folder. Watch out, it's loose.
(Whereupon, Deposition Exhibit Tuttle-28, Appendix C to Tuttle Expert Report, was marked for identification.)
BY MR. SOILEAU:
Q. This of course is Appendix C to your expert report in this case.
A. Yes, it is.
Q. Who prepared this document?
A. Myself and my support team.
Q. Step me through how it was prepared.
A. So generally speaking, we reviewed the Imerys and J&J documents as cited by Dr. Cook and Dr. Krekeler and tried to generate kind of a single table summary of those documents, ultimately culminating in a brief discussion of them in the report regarding specifically heavy metal analysis in the talcum powder samples.
Q. Okay. Let's see if I can break that down into a few questions that I have.
Is it correct that all of the source documents that led to the chart that is Appendix C came from Drs. Cook and Krekeler?
A. Yes, I believe.
Q. Did you obtain, request or gather any other documents other than documents that you had through Drs. Cook and

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Krekeler in order to be able to generate Appendix C?
A. No.
Q. Did you include in Appendix C all of the data available through the Cook and Krekeler documents?
MR. FROST: Objection.
A. I guess I'm not sure. I would need to look at all the data provided by Drs. Cook and Krekeler, compared with the documents we cite. As I said, we summarized them in regards to specific testing documents, so there may have been some they used that were used for alternative purposes that aren't included in this table. I'm not sure, because it's such a large number of documents.
BY MR. SOILEAU:
Q. You told me that Appendix C was prepared by you and your support team. Who took the lead in the preparation of Appendix C?
A. Again, it was my document and my summary of the reports. I believe that

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Dana Cubanski assisted me with the actual generation of the table.
Q. In terms of what information was to be included or not included in the table, who made that decision?
A. Again, this is -- it was my table. In regards to what was included or not included, that would have been my decision.
Q. And did you tell me that the primary purpose for Appendix C was to collect together in one document data that had been provided by Dr. Cook and then Dr. Krekeler?
A. Again, as I recall, it was to assess some -- the testing materials regarding talcum powder. In -- you know, in these documents, there's other products beyond just the consumer product of talcum powder that are referenced by Dr. Cook and Dr. Krekeler for the intents and purposes of some of the -- specifically the tables regarding heavy metals in that portion of my report.
Q. Have you ever conducted the

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type of testing that is necessary to generate the data that we see in the table that is Appendix C?
MR. FROST: Objection.
A. Throughout the table there are several different methods noted, depending on the document. There's -- and some of these methods I'm familiar with, some of these methods I have had samples analyzed for.
I have not done that type of analysis myself in regards to these samples, but again, there's several different methods in here, but some of them I have submitted samples of my own in the course of my work for analysis by similar methods.
BY MR. SOILEAU:
Q. So you have requested testing of materials at times in your work that would be of the same type of testing that we see reflected in some of these documents?
A. Yes, that's correct. As I said, there's a number of methodologies, some of which I have used in my own line of work.
Q. Yes, but I guess what I'm

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wondering, Doctor, you're not qualified to actually do the testing yourself. In other words, you request this information from someone, you receive the information and use it, but the actual performance of the test itself, is that something that falls within your qualifications?
A. I have done some of these methods over the course of my Ph.D. I don't do lab work in my work at CTEH at this time.
Q. You mentioned that you had a paragraph in your report that relied on Appendix C, I think. Is that at page 45? Take a look.
A. Yes, I believe that is the summary table that I was referring to.
Q. In the paragraph at the top of page 45 of your report, you say, quote, "In the case of crude talc ore, which had some of the highest detections for heavy metals, this is not comparable to the final product, which is processed via magnetic separation and acid washing to remove minerals and metals."
What is the basis for your

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conclusion that the talc referenced in the samples discussed in this paragraph had not gone through the steps necessary to bring it to a point to be comparable to the final product? In other words, what's the basis for this statement I just quoted from your report at page 45?

MR. FROST: Objection.

A. So in the -- going through the Imerys and J&J documents, it was clear that there was a wide variety of different products that were being assessed and sampled from ore materials down to baby powders.

And the reason this statement is there, and I cite the reference is that -- and it is my understanding, and again, I don't go into this in too great detail beyond what you see here, but that the materials as mined and the different grades do not represent what is present in the final form.

And in going through the materials, I tried to separate out the other samples that were, as I put in here, historical talc drill samples, mine sludge,

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grade 66 talc and other species from samples that stated baby powder or cosmetic talc.

Q. What steps had yet to be undertaken, in other words, what additional steps would this product still have undergone based on your understanding?

MR. FROST: Objection.

BY MR. SOILEAU:

Q. What was left to do?

A. As I said, I'm not versed on the step-by-step process of talc manufacturing. As I said, I cite this reference here that discusses it. I understand there is some processing that goes into generating a baby powder, but I did not research that in depth. I'm not versed in that process.

BY MR. SOILEAU:

Q. What is the basis for that understanding?

MR. FROST: Objection.

A. Well, again, I cite the Fiume study here that briefly states there is a process, and I'm aware there is a process and

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I know that there are others involved in this litigation that address that process and the difference, you know, between mined talc and talcum product in more detail than I do. I didn't assess that specifically for my purposes.

When looking through these documents, I tried to identify samples that were specific for baby powder that could be separated out from the other samples to look at the testing regarding heavy metals.

BY MR. SOILEAU:

Q. Have you attempted to identify the particular mines or sources for the talc that is used in Johnson & Johnson talcum powder products?

A. No, I have not.

Q. Do you have any education in geology?

A. Maybe some very cursory, but not an extensive education in geology, no. I'm not a geologist.

Q. Same question about mineralogist.

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A. I'm not a mineralogist, no.

Q. Okay. We had looked at a few documents from A.P. Wehner before we took our break. I'm going to show you one more that I've marked as Tuttle Exhibit 29.

(Whereupon, Deposition Exhibit Tuttle-29, 10/7/04 Wehner Letter, was marked for identification.)

BY MR. SOILEAU:

Q. This is a two-page document. It's a letter. It's addressed to Richard Zazenski, Z-A-Z-E-N-S-K-I. You may recognize his name from some of the other documents we looked at earlier today. And it is signed by Alfred P. Wehner.

Do you see in the first paragraph that he is referencing a copy of the retrograde migration paper by Sj?sten which has been added, he says, to the paper by Mills on perineal talc exposure and epithelial ovarian cancer risk?

A. Yes, I see that.

Q. I suppose you don't know if the Sj?sten, S-J-O-S-T-E-N, paper referenced here

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in the Wehner October 7, 2004 letter is the same paper I showed you today that you had not seen previously?
A. It doesn't specify the year, and again, these are personal communications so I have no way to know what they're discussing specifically.
Q. It says in part in here in the third paragraph: The study generally is very similar to 14 or so previous retrospective case-control studies with all the inherent well-discussed and published weaknesses of such studies. Its ORs are well below 2.0.
Do you see that part of the letter on the first page?
A. Yes, I see -- I see that part of the letter.
Q. Do you know what an OR is?
A. I believe it refers to an odds ratio.
Q. Okay. And what is the significance of an odds ratio that is below or well below 2.0?
A. In my training, odds ratios of

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2.0 was kind of the -- I'm trying to think of the best way to describe it, is the kind of established -- the level you start looking for when you're performing experiments.
As far as 2.0, you would like to see odds ratios above 2.0 in regards to looking at biological impacts or potential relationships.
Q. Does that mean that as long as you are at or below 2.0 in your opinion, you do not have evidence of a biological impact or potential relationship?
A. Well, as I said, 2.0 is kind of -- as I said, what I was taught and what we practice in our -- in my Ph.D. training and expertise as regards to when doing research and doing analysis of relationships, odds ratios above 2.0, you know, we kind of were -- it's rather hard to describe, but it's kind of the area we were looking for from a point of biological significance.
Q. Okay. Does that mean that taking the converse, that odds ratios that were not above 2.0, according to your

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training, did not have biological significance?
MR. FROST: Objection.
A. We're speaking in very broad terms. You know, it depends on the science and the data. You know, I'm speaking generally when I say that the odds ratio of 2.0 is kind of what we used when doing my research for my Ph.D. It's what was taught in -- when analyzing scientific data.
BY MR. SOILEAU:
Q. Are we in an area that is -- well, is this area comfortable to you, that is, within your expertise, comfortably within your expertise, when we start talking about odds ratios?
MR. FROST: Objection.
A. I'm familiar with odds ratios. I use odds ratios. You know, I'm familiar with confidence intervals, I have training and experience in them as part of my work in toxicology.
BY MR. SOILEAU:
Q. Right.

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The beginning of that statement sort of sounded like my plumbing. I used the plumbing. I'm familiar with the plumbing. I can do some things, but I'm not a plumber.
I'm just trying to understand if, you know, when we start talking about odds ratios, if that is an area -- and I'm going to take some letter -- I'm sorry, some language from this letter where he says recognized epidemiologists and statisticians.
I mean, is that who you would point me to or is this something that you feel you're well versed to discuss as an expert?
MR. FROST: Objection.
BY MR. SOILEAU:
Q. Odds ratios, that is?
MR. FROST: Objection.
A. I guess it would depend on the specific question. As I said, I have training and expertise in odds ratios and statistical analysis. I assess them, I use them. I'm not a statistician, so if there were things that we -- you know, getting into

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more detail regarding odds ratios or something, I would probably refer you to others that get more into the statistical analysis and the methodology surrounding statistical analysis.
BY MR. SOILEAU:
Q. When I asked you earlier about the converse of greater than 2.0 odds ratios, and I think you told me something like it's going to depend on some of the context or circumstances. You said you know it depends on the science and the data.
Is that sort of one of those areas where opinion comes into play, there's a subjective element to assess the data and decide if it is significant even though it's below 2.0?
MR. FROST: Objection.
A. No, that's not what I'm saying.
BY MR. SOILEAU:
Q. Okay.
A. I'm saying you need to know the context of the data itself to generally speak to an odds ratio, regardless whether it's

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above 2.0 or below 2.0, it requires more context about the science and the data.
Q. Based on your training, could an odds ratio of 1.6 be relevant, important scientific evidence on the issue of causation?
MR. FROST: Objection.
A. Again, it depends.
BY MR. SOILEAU:
Q. Well, could it ever? When I hear you say it depends, that means it might, it might not. So I'm just trying to be complete and fair. That means, yes, it could be, but, no, it might not be. And that's fine, but that's specifically what I'm asking.
Is it possible? You understand possible just means possible. Is it possible that under the appropriate circumstances an odds ratio of 1.6 could offer evidence of a cause relationship?
MR. FROST: Objection.
A. Again, it would -- it would -- it would depend. You'd have to look at the

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context. When we're talking about cause relationships, we always have to bring it back to the Hill criteria and the nine viewpoints as a whole.
As we said earlier, strength, which would be, you know, odds ratios is definitely a -- one of viewpoints of the Hill criteria and one of the key viewpoints, and as we talked about previously, when you're looking at them, you have to look at them as a whole and look at them in context when you're establishing your -- whether they support a causal association.
And odds ratio, again, you know, you need to look at the context of the study that developed the odds ratio, you need to look at other studies also as well to look at their odds ratios, and in general, look at the data to -- let's see what the data supports.
So as far as whether one particular odds ratio, you know, is or is not involved in causal, you have to look at the entire body of science in the context of the

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causal assessment.
BY MR. SOILEAU:
Q. So you couldn't just go, well, it's 1.6 or it's 1.4, 1.5, so I'm going to rule out any significance for that study?
You can't just out of hand do it; you have to look at the data, look at the study and decide what weight, if any, it's entitled to, fair?
MR. FROST: Objection.
A. Again, you have to look at the data and the science. You know, odds -- as I said, odds ratios are useful and there's -- we talked about the 2.0 or below 2.0, but you have to look at that as well as the scientific data as a whole.
BY MR. SOILEAU:
Q. Look at the second page of Exhibit 29. The middle paragraph includes a statement: I would recommend to have this study evaluated by a reputable epidemiologist well familiar with the talc issue (e.g., Dr. Huncharek, H-U-N-C-H-A-R-E-K, comes to mind).

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Do you recognize that name from your work in this litigation?
A. Yes, I believe so.
Q. How?
A. I think I cite some Huncharek -- I'm not sure if I'm saying it right -- references in my report.
Q. Do you want to check?
A. Certainly.
Q. Look at page 77, see if you see three different references.
A. Yes, I do.
Q. Do you know anything else about Dr. Huncharek?
A. No, I don't.
Q. Do you even know if he's -- well, the letter says he's a doctor, but you don't even know that, I guess.
A. When I look at scientific articles, I don't really research the authors. I look at the science and the data.
Q. Do you look at the publication to see where it was published?
A. Obviously when citing them and

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stuff, I look at the journals where they have been published, but I don't necessarily, as far as assessing the journals themselves.
Q. Do you still maintain as we sit here this afternoon that there is no scientific evidence to support the theory of migration and that Drs. Plunkett and Zelikoff were scientifically unsound and flawed in their opinions?
MR. FROST: Objection, misstates testimony, misstates opinion.
A. Well, again, I would refer to the opinions in my report. The scientific evidence does not support the hypothesis that external application of talcum powder can migrate through the female reproductive tract to the ovaries, and in the opinions of my report, I do not criticize Dr. Plunkett or Dr. Zelikoff. I merely am critical of the methodologies, and as I said, the scientific evidence does not support the hypotheses.
BY MR. SOILEAU:
Q. Does that mean that you stand

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by what you said in your report on these issues, migration?
A. Yes, my report -- my report is my work, and I stand by what I write in my report, yes.
Q. And nothing that we've discussed today causes you to want to pause and revisit any of your opinions or analysis of the migration issue?
A. Well, as I've said before, you've -- you've shown me some personal correspondence that would have no bearing on a scientific investigation, and the articles you've provided me, I haven't had a chance to read in depth. But generally speaking, none of them looked at external perineal application and the successful migration from external application to the ovaries.
Q. So you do not feel as we sit here today any need to say I'd like to revisit that issue?
A. Well, as I state in my report, you know, if new evidence comes available, I always reserve the right, but based on what

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you've presented to me and what I have seen in the scientific evidence, there's nothing to support the proposed migration theory of external application of talcum powder migrates to the ovaries.
Q. The issue of plausibility, it includes -- the plausibility under the Hill viewpoints, that includes mechanism, right?
A. I -- the biological plausibility -- we can refer to the Hill to look at it distinctly, but yes, it generally refers to a proposed mechanism of exposure or of toxicity.
Q. Okay. So a proposed mechanism of toxicity.
What does the word "toxicity" mean?
A. Of adverse health effect, how the adverse health effect is caused by a certain dose of product.
Q. Okay. And will you agree that investigators have studied proposed mechanisms by which talc might initiate or contribute to a carcinogenic process?

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MR. FROST: Objection.
A. I am aware there have been some mechanistic studies regarding talcum powder and ovarian cells in vitro and in vivo.
BY MR. SOILEAU:
Q. Well, let me ask you this: Can you agree with this statement: Investigators have studied proposed mechanisms by which talc might initiate or contribute to a carcinogenic process?
MR. FROST: Objection.
BY MR. SOILEAU:
Q. Can you agree with that?
A. Again, I'm aware that research has been done both in vitro and in vivo. I do not get into that in my report. I don't do a detailed research regarding the genetic mechanistic research regarding talcum powder and ovarian cancer.
Q. Would you look at your report at page 27, please, Doctor? Do you find there a one-paragraph section numbered 7.3.3 and entitled Studies on Talc, Inflammation and Ovarian Cancer?

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A. Yes, I see it.
Q. Would you read that statement, the first sentence of that paragraph into the record, please?
A. Investigators have studied proposed mechanisms by which talc might initiate or contribute to a carcinogenic process, including whether talc causes inflammation or whether inflammation is itself related to the initiation of ovarian cancer.
Q. So, Doctor, let me ask you again. Can you agree with this statement: Investigators have studied proposed mechanisms by which talc might initiate or contribute to a carcinogenic process?
MR. FROST: Objection.
A. Again, that's what I state in the first half of that sentence of my report, and as I stated previously, I'm aware that there have been in vitro and in vivo studies looking at genetic mechanisms.
BY MR. SOILEAU:
Q. Precisely, the statement is

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taken verbatim from your report, right?
MR. FROST: Objection.
A. Again, it's the first half of that sentence in the paragraph discussing studies on talc, inflammation and ovarian cancer.
BY MR. SOILEAU:
Q. How about this: The words that I presented to you are, in fact, your words, Doctor.
MR. FROST: Objection.
A. Again, as I stated, that is what I've written in the first half of that sentence on that paragraph regarding on studies on talc, inflammation and ovarian cancer.
BY MR. SOILEAU:
Q. Right. That's why I say these words are your words, fair?
MR. FROST: Objection.
A. Again, that's the first half of the sentence that is written there, along with the rest of the paragraph.
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BY MR. SOILEAU:
Q. I mean, you don't suggest I have to read the whole report to you for it to be your words. I'm not saying that's the entirety of your report. I'm just saying -- well, did you write those words?
A. Yes, I wrote those words.
Q. Okay. So they are your words?
A. Again, yes, those are the words that I wrote, that is the first half of that sentence in that paragraph.
Q. Well, once we get past this, I'm going to keep going on the sentence where we got hung up. Let's see if we can keep going.
And when investigators have studied proposed mechanisms by which talc might initiate or contribute to a carcinogenic process, can you agree that has included whether talc causes inflammation?
A. I'm sorry, can you repeat that for me?
Q. Sure.
A. I was -- I thought you were

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quoting my report.
Q. Well, I sort of took it from your report, but I'm simply asking you if investigators who have studied proposed mechanisms by which talc might initiate or contribute to a carcinogenic process have included within those studies the question of whether talc causes inflammation?
A. And again, I'm aware of studies that have looked at whether talc causes inflammation.
Q. And also whether inflammation is itself related to the initiation of ovarian cancer, fair?
A. Yes. Again, I'm aware that investigators have studied that.
Q. You will agree with me that when large numbers of foreign particles of any composition deposit on tissue, an initial response can be irritation?
MR. FROST: Objection.
A. Well, you're speaking in broad strokes --
///

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BY MR. SOILEAU:
Q. Yes.
A. -- again, and, you know, as you said, can cause inflammation. Depending on the scenario, the exposure, the particles, yes, acute inflammation can occur.
Q. Well, isn't the initial response irritation?
MR. FROST: Objection.
A. We were just discussing inflammation, so now we jumped to irritation. Is there -- I'm afraid you're kind of -- I'm not sure how you define irritation versus inflammation.
BY MR. SOILEAU:
Q. Well, I was just asking, if you have a large number of foreign particles of any composition deposit on tissue, if the initial response is irration? Go ahead. You want to answer it or you want me to rephrase it?
A. I was going to say it depends.
Q. Let's put it in the lungs. You've done a lot of work on inhalation

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issues, haven't you?
A. Inhalation toxicology is something we do a lot of at CTEH, yes.
Q. Right. And the work that you've done on asbestos and some chemical releases that are referenced in some of the materials that I've been provided with or gathered, a lot of those involve inhalation; not suggesting that it's limited to just inhalation, but inhalation has been an issue in a lot of those projects that you've worked on?
A. Yes.
Q. Okay. If large numbers of foreign particles of any composition deposit in the airways, will the initial response be irritation?
A. Again, it depends. Depends on the foreign particle, depends on the dose, but certainly there are circumstances where foreign bodies may deposit on the lung and cause irritation.
Q. And it's possible that irritation will be followed by inflammation?

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A. Again, it depends on the scenario and the dose and a number of other factors. Again, when you're talking about the lung, there are certainly conditions for which foreign bodies will deposit in the lung and cause inflammation, an acute inflammatory response.
Q. Okay. And if you get an inflammatory response, are there macrophages that then play a central role?
A. There are a large number of different components involved in the inflammatory process, and I have not refreshed my memory recently enough to be able to recall all of them involved. Macrophages are certainly involved.
Q. Well, isn't it fair to say that the macrophages in the lungs in the respiratory system would play a central role if you had a deposition of foreign particles that triggered irritation followed by inflammation?
MR. FROST: Objection.
A. As I said, there's a large

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number of different cells and cytokines and things involved in the inflammatory response. I can't say whether macrophages play a central role without refreshing my knowledge about the inflammatory response and the individual cells involved in it.
BY MR. SOILEAU:
Q. Earlier you spoke about defense systems, I believe, in the body. Is it true that different organs have different levels, different degrees of defense systems?
A. Again, that's a very general statement. As far as -- as I said previously, the body has a number of different defense mechanisms.
Q. But are the body's defense mechanisms, if you know, the same for different organs? For example, the same in the lungs as in the kidneys as in the ovaries as in the brain, or does it vary from organ to organ, if you know?
A. Yeah, I have not done a detailed assessment of the different target organs' defense mechanisms. I would say that

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there are some that are the same and some that vary. But we would have to kind of compare -- pick two organs and actually compare the defense mechanisms.
Q. Lungs and ovaries.
A. Well, again as I said, I haven't done a detailed research for every defense mechanism and I said earlier that I have not studied specifically the defense mechanisms of the ovaries.
Q. Okay. Are we, from your perspective, still within your area of expertise?
MR. FROST: Objection.
A. Well, as I said, you know, I address inflammation briefly in my report. I do not get into the detailed mechanisms of inflammation. As I said previously, I have not researched the defense mechanisms of the ovary. I don't address the defense mechanisms of the ovary in my report.
BY MR. SOILEAU:
Q. Do you know what the word phagocytize means?

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A. Yes, I believe so.
Q. Does that involve the effect or process of the macrophages in the body, if we go back to our foreign particles in the lungs, the macrophages' response to these foreign particles in the body?
MR. FROST: Objection.
BY MR. SOILEAU:
Q. Is that generally what we're talking about?
A. And again, as I said, I haven't brushed up on my knowledge of the detailed -- the complex inflammatory process. Phagocy -- phagocytize, I'm sorry if I am not saying 1999 right, but --
Q. I had to practice. Phagocytize.
A. I don't recall and can't say that it's specific to macrophages, but it certainly involves the uptake of material, generally speaking, into cells, and not necessarily foreign bodies. It can be involving other products as well.
Q. This process that I'm sort of

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stepping through here, foreign particles, irritation, inflammatory responses, macrophages, phagocytize, other defense mechanisms, this is a process that can unfold in the body when it's confronted with some foreign agent. You know that as a toxicologist, right?
MR. FROST: Objection.
A. Inflammation can occur in the body for a number of reasons. It's a healthy response in the human body beyond just foreign bodies.
BY MR. SOILEAU:
Q. Right. But it can also be a response to foreign particles, can it not?
A. Again, depending on the situation and the dose and stuff, there are certainly instances where foreign bodies can induce acute inflammation.
Q. Okay. And it can trigger what we would call a cascade, that is, a cascade of responses and reactions within the tissue, if the dose is sufficient to trigger toxicity?

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MR. FROST: Objection.
BY MR. SOILEAU:
Q. The dose of the foreign particle, right?
MR. FROST: Objection.
A. You're getting a little out of what I cover in my report and what I have done. As I said, I hav[illegible]n't done a refresher course on inflammatory response. That would depend, because depending on the chemical and the exposure of issue, you know, as far as whether the inflammatory response is involved in the mechanism of toxicity.
So I don't -- I can't speak to that specifically without doing some additional research, having a specific scenario.
BY MR. SOILEAU:
Q. Do you -- I'm sorry, I didn't mean to step over your words.
Do you recognize the term "cascade," Doctor, in this context?
A. Yes, but as I said, we're getting a little out of the realm of what I

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discuss in my opinions.
Q. In some scenarios -- I understand that. You do have a section on inflammation and talc, but you haven't discussed some of these issues that I'm bringing up that center upon the body's response to foreign particles and inflammation and this cascade, fair?
MR. FROST: Objection.
BY MR. SOILEAU:
Q. That's what you're telling me right?
A. Again, yes. As I said, you're discussing the cascades and the intricate mechanisms of the inflammatory responses is not something that I do in my report and not something that I research in depth in my report. I believe there are others who get more into the mechanisms and pathways regarding inflammation than I do.
Q. Well, is it something that you've written on?
A. You mean in my report, or do you mean generally or --

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Q. Generally.
A. I think I --
Q. Let me show you -- go ahead, I'm sorry, you have an answer?
A. Yeah, I was going to say I think I have done some inflammation work in my Ph.D. I can't remember if I specifically addressed it in one of my publications or not. But as I said before, I have -- I'm aware of inflammation, but I haven't brushed up on the intricate genetic roles and all the complexities of it.
Q. Let me show you an article that I read and make sure it's one that you are an author -- one of the authors of.
(Whereupon, Deposition Exhibit Tuttle-30, 2014 Nony et al Publication, was marked for identification.)
BY MR. SOILEAU:
Q. I've marked it as Tuttle Exhibit 30. Do you recognize this article?
A. Yes, I do.
Q. Are you, in fact, one of the

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three authors of this article?
A. I am.
Q. Would you turn to page 2, Mechanisms of Toxicity? You see it?
A. Yes.
Q. It does say: When large numbers of foreign particles of any composition deposit in the airways or the bronchial alveolar region of the lung, the initial response is irritation. Doesn't it?
A. Yes, that's what it states right here, it says the initial response is irritation followed by inflammation.
Q. And it says: Followed by inflammation in which the AMs, that's capital A, capital M, little s, play a central role. Do you know what the AMs are there?
A. If I remember correctly, and I'm sure we probably elucidated earlier, I believe it refers to activated macrophages.
Q. Okay. It could be -- I can't say the word very well. Alveolar?
A. Alveolar?
Q. I tell you what. Go up to the

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paragraph before, right in the middle, and let me show you on the screen. You see right here?

MR. SOILEAU: Oops.

(Comments off the stenographic record.)

BY MR. SOILEAU:

Q. So what does AM stand for?

A. Alveolar macrophages.

Q. Alveolar, thank you. Now I can say it correctly.

And then it goes on to say, still under Mechanisms of Toxicity near the bottom of the page: The AMs migrate to sites of dust deposition and attempt to phagocytize the particles, destroy them or translocate them to the -- what's that word?

A. Mucociliary.

Q. Escalator.

What does that mean?

A. So it's one of the defense mechanisms that we were discussing, and again, it's been a few years since this was drafted, so I will try to speak accurately.

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Q. Okay.

A. But when you think of -- to put it in layman's terms, coughing up a loogie.

Q. Okay. Right. This is one of the things the respiratory system can do to expel things that have come into it, foreign things that have come into the respiratory system?

MR. FROST: Objection.

BY MR. SOILEAU:

Q. Is that right?

A. Again, it's one of the defense mechanisms.

Q. Yeah.

A. In this particular chapter where we're referring to foreign particles, I think it can also be used in other scenarios within the lung as well.

Q. Sure. Let me finish this paragraph.

Simultaneously, the phagocytes release a complex series of chemical messengers that activate and attract inflammatory cells stimulating mucous

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secretion to generate -- generation of reactive oxygen species (ROS), and release of inflammatory mediators.

What are reactive oxygen species?

A. So I don't know that I can really define that very clearly just, as I said, off the cuff. You know, reactive oxygen species, you know, I probably prefer to refer to a definition in front of me to make sure I'm speaking accurately regarding them.

Q. Okay. Do you know if reactive oxygen species are significant to the carcinogenic process?

MR. FROST: Objection.

A. It would depend. I have not done a specific assessment of that in this case, and it would depend. I'd need more information or I'd need to be able to research that.

BY MR. SOILEAU:

Q. Let's look at the last sentence: The cascade continues as long as

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the irritant particles persist, subsiding only when and if the particles are cleared from the lung.

Do you agree with that statement?

MR. FROST: Objection.

A. Well, again, as I said before, when exposed in the lung, as I stated previously, foreign particles at a sufficient dose can certainly cause irritation followed by inflammation.

As far as the cascade statement in this particular context, looking at this, you know, the response is there to remove these foreign particles, so as long as the particles are there, and then when they're removed, the inflammatory response ends.

Cascade, again, what you asked earlier involved cascade and toxicity, and this is a little bit of a different scenario that we're looking at in this article, just the general inflammatory response.

BY MR. SOILEAU:

Q. Well, this is not discussing

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mechanism of toxicity in this article?
A. Again, yes, this is discussing mechanism of toxicity very generally in regards to foreign particles.
Q. That's what I thought. That's what I understood.
Let's go back for a moment to Exhibit 23, that FDA response that we discussed earlier. I'm going back to page 5 of 7. I'm revisiting language we looked at earlier.
I'm going to go to the middle of that paragraph. It is, therefore, plausible that perineal talc (and other particulate) that reaches the endometrial cavity, fallopian tubes, ovaries and peritoneum, may elicit a foreign body type reaction and inflammatory response that, in some exposed women, may progress to epithelial cancers.
You see that language that I just read?
A. Yes. Again, that's one sentence in that paragraph that I believe

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we've revisited -- we visited previously.
Q. Isn't the foreign body type reaction and inflammatory response that is noted here in the FDA paper from 2014 referring to the same sort of foreign body type reaction and inflammatory response that you discuss in Tuttle Exhibit 30 under Mechanism of Toxicity?
MR. FROST: Objection.
A. I don't know. As previously stated, this is a letter. They don't cite references. We're talking about the ovaries and other avenues of the female reproductive system compared to the lung, which is what we were discussing in this synthetic vitreous fibers study.
So as we said before, the inflammatory response is diverse and complicated, so, no, I don't know.
BY MR. SOILEAU:
Q. What about from Exhibit 22, which is the cover transmission to Mr. Ashton from Zaren- -- Zazenski, I'm sorry, Z-A-R-E-N-S-K-I -- a name we saw in another

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document.
When he says in this paragraph, in part: Combine this evidence with the theory that talc deposition on the ovarian epithelium initiates epithelium inflammation.
Is that in the same genre as the same thing, the same mechanism that we've talked about in each of these articles where there's a foreign body present on tissue and the reaction of the tissue is inflammation?
MR. FROST: Objection.
A. Well, again, as I said previously with the FDA letter, this is the personal correspondence. He refers to a theory. Without more information about what is he referring to, I can't speak to what he is -- what is he speaking to.
BY MR. SOILEAU:
Q. But that is what you said in the article that you helped write, Tuttle Exhibit 30, an inflammatory process that you describe under Mechanism of Toxicity. You know what that means.
You helped write -- you signed

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off on Mechanism of Toxicity under article -- I'm sorry, under Tuttle Exhibit 30.
MR. FROST: Is there a question? Objection.
BY MR. SOILEAU:
Q. You're telling me that the FDA letter and Exhibit 22 that I've shown you, which talk about inflammation, that you don't -- you don't seem to know enough about them to comment on them. And I was just asking, you know enough to comment about Exhibit 30, your article, don't you?
MR. FROST: Objection, misstates testimony.
A. Well, again, as I said, we wrote this, you know, several years ago, looks like in 2014, and, you know, we're speaking very generally about the inflammatory process in the lung in this particular area.
As we discussed previously, when looking at the defense mechanisms of the body, I have not done -- I've not compared all the organs of the body and their various

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defense mechanisms, and I think I've said several times that the inflammatory response is very complex and there's a lot of different cells and cytokines and things involved, which we don't discuss in this Mechanisms of Toxicity. It's a very general paragraph that we're discussing.

And I have not, I think I said earlier, brushed up on my understanding of the mechanistic toxicology or the mechanisms revolving inflammation in regards to my report or the assessment of talcum powder and ovarian cancer.

BY MR. SOILEAU:

Q. Well, given that, Doctor, isn't it is it fair to say that you cannot offer opinions on the existence or nonexistence of a plausible mechanism for ovarian cancer that involves foreign particles, inflammation and a cascade of events that results in carcinogenicity?

MR. FROST: Objection.

BY MR. SOILEAU:

Q. Well, you're not in a position

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to comment on that today?

MR. FROST: Objection.

A. There were --

THE WITNESS: I'm sorry.

A. There were multiple parts to that question. Can we break it --

BY MR. SOILEAU:

Q. Right. But they all go back to what we're talking about.

Isn't it fair to say, Doctor, that you're not in a position today, you do not have a foundation from which to offer an opinion on the existence or nonexistence of a plausible mechanism for talc particles to trigger ovarian cancer?

MR. FROST: Objection.

A. So that's a -- that's a different question than what you were asking previously.

BY MR. SOILEAU:

Q. It is, but you've told me today -- let's back up for a step or two and I'll lay it out better.

You've told me you've not

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looked at the defense mechanisms of the ovary, correct?

MR. FROST: Objection.

A. I said I've not done research into the defense mechanisms of the ovary or any, you know, specifics in regards to the defense mechanisms of the ovary.

BY MR. SOILEAU:

Q. You looked at migration, right?

A. I looked at the scientific evidence of whether there was any support for the theory of external migration from perineal application of talc to the ovaries.

Q. And you found no evidence?

A. That's correct, the scientific evidence does not support the migration theory.

Q. You did not look at evidence involving the existence of talc within ovarian tissue, did you?

MR. FROST: Objection.

A. As I think we discussed previously in the -- as far as the presence of talc in ovarian tissue, we -- in regards

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to the migration theory, I looked at scientific evidence on whether there was any evidence to support the theory that external application of talc would migrate to the ovaries.

BY MR. SOILEAU:

Q. Do you have an opinion, Doctor, as to whether there is evidence to support a potential mechanism for talc to cause ovarian cancer following perineal application?

MR. FROST: Objection.

A. As I state in my report when looking at the biological plausibility in the Hill criteria, I base my scientific evidence of the migration theory that the talc will reach the ovaries based on perineal application for which the scientific evidence doesn't support that.

BY MR. SOILEAU:

Q. Here's what I hear you telling me, and you've been over it a few times, many times today, and that is, you do not see scientific evidence to support the ability of talcum powder applied to the perineum to

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migrate to the ovaries. Therefore, you've not looked at any mechanistic issues involving talc reaching the ovaries because you don't believe it happens.
MR. FROST: Objection.
BY MR. SOILEAU:
Q. And I'm just trying to figure out if you have an opinion, one way or another, about any potential mechanism. And from what you've told me, it wouldn't seem like you had developed a foundation from which to speak to that.
MR. FROST: Objection.
A. Well, as I said, I didn't do an extensive review regarding the genetic mechanisms of inflammation, and again, inflammation is not the same as the initiation of cancer.
And so, you know, I did not do a detailed review regarding the inflammatory process. We talked -- we quoted a few sentences from the paragraph that I have on page 27 where I look briefly at some of the studies that look at talc inflammation and

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ovarian cancer, but I have not done a detailed assessment of the inflammatory process or the genetic mechanism; therefore, I think there are others that do that.
BY MR. SOILEAU:
Q. It can be though, right?
MR. FROST: Objection.
A. I'm sorry?
BY MR. SOILEAU:
Q. It can be. Inflammation can be a step in the development of cancer?
MR. FROST: Objection.
BY MR. SOILEAU:
Q. Isn't that true?
MR. FROST: Objection.
A. I would need more information because I think you're referring to promotion as opposed to initiation.
BY MR. SOILEAU:
Q. I was just asking if inflammation can be an initial step in the body that ultimately develops into a cancer.
MR. FROST: Objection.
///

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BY MR. SOILEAU:
Q. Do you know?
A. Again, I --
MR. FROST: Objection.
A. I am aware generally that there are a very few number of cancers for which initiation [sic] may be involved in the initiation process. I think that there's somebody else, another expert, that discusses that specifically.
In my report when I discussed carcinogenesis, inflammation is more associated with promotion rather than initiation.
BY MR. SOILEAU:
Q. Today is my opportunity to talk to you. Should I expect that you would come into the courtroom and offer an opinion for or against the existence of a plausible mechanism for the development of ovarian cancer triggered by talc on the ovaries?
MR. FROST: Objection.
Objection.
A. I'm sorry, can you...

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BY MR. SOILEAU:
Q. I'm just trying to figure out if you have an opinion one way or another on the question of: Is there a plausible mechanism for talc to produce or contribute as a significant factor to the development of ovarian cancer?
MR. FROST: Objection.
A. So again, you know, my opinions in my report are stated, and I briefly touch upon inflammation. That being said, my -- the scientific evidence does not support a causal association between talc and ovarian cancer. In regards to a plausible mechanism, we already discussed migration in depth. I don't, I believe, get into migration or, as I said, the mechanistics of inflammation in great detail in my report.
I look at the scientific literature very generally. There are others that get more in depth as far as mechanisms than I do.
BY MR. SOILEAU:
Q. Well, you've just got that one

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paragraph on inflammation, right?
MR. FROST: Objection.
A. I would need to go through. As I said, I think in the carcinogenesis portion of my report, I discuss inflammation briefly as well in a general role --
BY MR. SOILEAU:
Q. Right.
A. -- regarding carcinogenicity.
Q. That's true. Right. And that's fair.
I meant in the context of talc, you only have this one paragraph at page 27, 7.3.3.
A. I -- as I said, I don't get into the mechanisms of inflammation in great detail. I do believe that in discussing fragrant chemicals, there's also some references to irritation and potentially inflammation, but without doing a quick word search on it, I can't speak that this is the only place that I mention inflammation other than what we discussed in the --
Q. Okay. Just to be clear, I

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didn't really -- I really meant specifically inflammation and talc. I know that inflammation is discussed elsewhere when you talk about carcinogenicity.
What is cytotoxicity?
A. Goodness. That is --
Q. Did I step out of your area?
MR. FROST: Objection.
A. I'm not comfortable answering it off the cuff. I'm not sure that I can define it very clearly and scientifically.
BY MR. SOILEAU:
Q. What relevance, if any, does cytotoxicity have for discussion like the one we're having today?
MR. FROST: Objection.
A. Again, we're getting a little out of what I did in regards to my report and what I researched for --
BY MR. SOILEAU:
Q. Okay. All right.
A. -- the scientific literature.
Q. Are we getting out of your area of expertise?

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MR. FROST: Objection.
A. As I said, I have experience and expertise in inflammation and researching inflammation. As I said previously, I did not research -- brush up on the mechanisms of the -- the inflammatory process and the mechanisms, I don't get into that, into my -- in my report in this case.
BY MR. SOILEAU:
Q. Is talc cytotoxic?
A. I don't know.
Q. Okay. Let me show you an exhibit I will mark as Tuttle Exhibit 31.
(Whereupon, Deposition Exhibit Tuttle-31, 2/17/77 Schneider Letter, was marked for identification.)
MR. SOILEAU: The only copies we have, for the record, have some highlighting on them, and we've not been able to get that off. So every copy will have the same highlighting. It's not highlighting that I applied. And I really suspect you guys know that already. What that's?

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MR. FROST: I believe I've seen this highlighting before.
MR. SOILEAU: Yes. I thought as much.
MR. FROST: Is this 31?
THE WITNESS: Yes.
MR. FROST: I believe this was highlighted once for trial.
MR. SOILEAU: I think everyone in the room knows more about these exhibits than I do, so I appreciate that. Trying to keep up.
BY MR. SOILEAU:
Q. Do you see the name Battelle at the top?
A. Yes, I do.
Q. The third page of this three-page document, it's signed by R.P. Schneider, Ph.D., assistant manager, molecular biology and biophysics.
Do you recognize that name?
A. Not off the top of my head, no.
Q. It begins saying -- what about William Sherman? I'm sorry, William Sherman,

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Ph.D., manager of biomedical evaluations, Johnson & Johnson Baby Products Company.
Do you know who that is?
A. No, I don't.
Q. Okay. And this goes all the way back to February 17, 1977.
It says: Dear Bill, as I related to you on the telephone, we have spent more time studying the effects of talc than we anticipated. Since we have found a cytotoxic effect, I felt that we should pursue it in more detail and obtain data which can be compared to other studies. This has required doing more short-term incubation experiments in the absence of serum.
Is a cytotoxic effect for talc significant to you as a toxicologist?
MR. FROST: Objection.
A. It depends. I would need more information. I would need to see the studies that they're discussing. You know, this is, again, a personal communication. This isn't a research article where they're discussing the studies that they're performing.

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BY MR. SOILEAU:
Q. Okay. But I'm simply asking you a general question right now, Dr. Tuttle.
If talc is cytotoxic, is that relevant to your opinions in this case?
MR. FROST: Objection.
A. Again, you know, my opinions are -- you know, cytotoxic, again, it would depend on these studies and what information is being shown. But as I said previously, I assessed the scientific literature regarding the -- whether the exposure to talcum powder perineally, what -- the scientific evidence showed a causal association between perineal talcum powder exposure and ovarian cancer.
BY MR. SOILEAU:
Q. We said at the beginning of our discussion today, and I think we agreed that toxicology includes the study of adverse effects of agents on organisms, right?
A. Generally speaking, yes, the science of toxicology is the study of potential adverse health effects relating to dose and exposure metrics on living

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organisms, including the environment.
Q. And at a most basic level, doesn't cytotoxic mean that it is toxic -- the agent or substance is toxic to the cells of the body?
MR. FROST: Objection.
A. Again, as I said earlier, I'm not sure that I can accurately define cytotoxicity just off the cuff without referring to a document.
BY MR. SOILEAU:
Q. Okay.
A. I don't want to use layman terms and then be inaccurate or misspeak.
Q. I can show it to you if we need to, but the Wehner document, one of the Wehner documents we looked at earlier, talked about intended or normal use of talcum powder products.
Have you seen that phrase in your work on this project, intended or normal use of talcum powder products?
A. I didn't specifically look for that phrase in my assessment of the

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scientific literature, so I couldn't say.
Q. Do you have an understanding whether genital application of talcum powder products is considered a normal and intended use of the product?
MR. FROST: Objection.
BY MR. SOILEAU:
Q. I'm just asking if you have an understanding.
MR. FROST: Objection.
A. I'm -- I don't know. As I said, I didn't look for that phrase in my assessment of the scientific literature. I merely looked at whether the scientific evidence for the perineal use of talcum powder, if the scientific evidence supported a causal association with ovarian cancer.
BY MR. SOILEAU:
Q. But you did not consider the possibility that talc was cytotoxic, correct?
MR. FROST: Objection.
A. Well, again, in looking at whether there is a causal association when you're looking at the epidemiological studies

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or, you know, the study cited in the report, you know, looking for that causal association, as I said before, I didn't get into the genetic mechanisms as far as we discussed with inflammation or cytotoxicity, but looking at the body of evidence to see whether there is a causal association.
BY MR. SOILEAU:
Q. Back on the Health Canada study for just a moment. I think we discussed that you had cited it in your report, of course, the draft assessment?
A. Yes. Can you refer me to which exhibit number it is?
Q. I can. My question is more general, but I'm glad to tell you which exhibit number it is.
I'm going to tell you my question is: Are you familiar with the methodology that Health Canada uses?
I think you'll find that it's Exhibit 21.
A. Thank you.
And can you repeat your

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question for me, please?
Q. Sure. Are you familiar with the methodology that Health Canada used in preparing the draft assessment?
A. I am not specifically familiar with the methodology. As I look at the assessment, I would need to refamiliarize -- I think that they go through their methodology in other documents on their website.
Q. I think so. I think you're correct.
As we sit here today, do you have any criticism of the methodology used by Health Canada in its draft assessment?
MR. FROST: Objection.
A. Well, as I said before, without having the other documents where they discuss their methodology in detail, I can't discuss it.
BY MR. SOILEAU:
Q. Let me show you a document I've marked as Exhibit 32, Tuttle Exhibit 32.
(Whereupon, Deposition Exhibit

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Tuttle-32, Health Canada Risk Assessment Framework Study, was marked for identification.)
BY MR. SOILEAU:
Q. Have you seen this before? It says Risk Assessment Framework Summary, and it's -- well, it speaks for itself.
Have you seen that before or do you know?
A. No, I don't believe I have.
Q. I'm really just asking whether, as we sit here today, having had the opportunity to look at the Health Canada draft assessment, if you have any criticisms of their methodology or the manner in which they applied their methodology to the evidence that they looked at?
MR. FROST: Objection.
A. Well, as I said, I -- the methodology -- I haven't reviewed the Exhibit 32 that you provided to me before. I haven't seen it, so I can't discuss the methodology that Health Canada used without some time to look at the methodology.

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BY MR. SOILEAU:
Q. You had not considered the actual methodology used by Health Canada when you considered their draft assessment on talc?
MR. FROST: Objection.
A. As I said, in their draft assessment, they don't really go into their methodology. As I said, I think it was present elsewhere on their framework, but I --
BY MR. SOILEAU:
Q. My question -- I'm sorry, go ahead.
A. No, but I did not review their methodology for their draft screening assessment.
Q. Right. That really was my question, that in connection with your review of that document, you did not determine the methodology.
MR. FROST: Objection.
A. I -- again, I didn't review the methodology that they used.

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BY MR. SOILEAU:
Q. Okay. So you would not have any criticisms of it, obviously, because it's not something you've looked at, fair?
MR. FROST: Objection.
A. Again, I would need to look at it to be able to discuss it.
BY MR. SOILEAU:
Q. Okay. You say in your report -- turning to your report, at page 34. Let me let you find that. I'm looking at the fourth paragraph, the second sentence right after footnote 4: This by definition renders the products tested as nonasbestos-containing.
You see that?
A. Yes, I see that.
Q. And then you cite a document, United Nations, 2017?
A. Yes.
Q. All right. Let me show you a document that I have here.
THE WITNESS: If I may, while he's doing that, how long have we been

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going?
THE VIDEOGRAPHER: One hour and 10 minutes.
MR. SOILEAU: I only have two copies of this. I'm going to give one to the witness and one to counsel to sort of share.
MR. FROST: Sure.
MR. SOILEAU: Let me go ahead and put a number on it. This will be identified as Tuttle Exhibit 33.
(Whereupon, Deposition Exhibit Tuttle-33, Global Harmonized System of Classification and Labeling of Chemicals, was marked for identification.)
MR. SOILEAU: I could not find a way to make this smaller under the circumstances.
BY MR. SOILEAU:
Q. My question, Doctor, my first question applicable to this exhibit, Tuttle Exhibit 33: Is this the exhibit that you reference at page 34 of your report when you

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say: This by definition renders the product tested as nonasbestos-containing?
A. It is one of the references that I cite in that paragraph regarding asbestos-containing, yes.
Q. Well, it is the -- let's be more specific.
The sentence that I read: This by definition renders the products tested as nonasbestos-containing (United Nations, 2017).
So there's one citation and only one citation, and it is part of that sentence, that is, the parenthetical is inserted before the period, agreed?
A. For that specific sentence, yes.
Q. Okay. And so my question to you is whether Tuttle Exhibit 33 that I have marked and presented to you here today is, in fact, the United Nations 2017 document that you reference in that sentence on page 34 of your report?
A. Well, the cover page says that

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it is. It is a large and sizable document, so I don't know if it's here in its entirety. But the cover page says it is the Globally Harmonized System of Classification and Labeling of Chemicals.
Q. All right, Doctor. Are you familiar with that document?
A. Yes.
Q. For example, have you used it before?
A. Yes, I've used this document.
Q. Do you maintain, Doctor, that this exhibit, Tuttle Exhibit 33, the United Nations document, includes a definition of the term "nonasbestos-containing"?
A. No, I don't believe it provides a verbatim definition of nonasbestos-containing.
Q. Do you know if the term "asbestos" or the words "asbestos-containing" appear anywhere in the document?
A. No. I -- I don't know specifically. I would have to do a check if they reference asbestos-containing in the

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document, but in regards to, you know, the classification and labeling as far as the information provided, I don't believe so.
Q. I can only tell you that I did and I couldn't find those words, and I'm not going to ask you to do it right now.
But why do you reference a definition citing this paper if there is no actual definition of nonasbestos-containing, that terminology, in this document?
MR. FROST: Objection.
A. So again, this is one of the references I cite, and the reason I cite it is when you go through the Global Harmonization Standard, it is a standard that, as it states on the label, provides the guidelines that the United Nations and OSHA, the Occupational Safety and Health Administration, have put forth regarding classification and labeling of chemicals.
And the reason that that is important -- and I think I discuss it in more detail in other parts of my report -- actually, I do at the bottom of that same

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page, they provide information regarding substances and concentration limits in regards to hazard labels, hazard identifications, and for -- in this particular case we're referring to asbestos, and they provide for carcinogens a .1% cutoff in regards to labeling or including a material as being present in a compound on an MSDS or on a hazard label or identification label.
BY MR. SOILEAU:
Q. Is asbestos a carcinogen?
A. IARC has classified asbestos as a known human carcinogen.
Q. Is asbestos a known carcinogen for ovarian cancer?
A. I believe I discussed this in my report. I believe IARC found that the heavy occupational levels of asbestos exposure associated with ovarian cancer, I can't remember offhand if they also classified it as a Group 1, but they certainly said that heavy occupational exposures are associated with ovarian cancer.

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Q. I appreciate that. I'm really -- I was asking you whether, as a carcinogen, asbestos included -- or it was determined that asbestos caused, among the cancers it causes, ovarian cancer?
MR. FROST: Objection.
MR. SOILEAU: Yeah, that's not really a question, so it's okay.
BY MR. SOILEAU:
Q. I'm still a little lost -- I guess we can just agree on this: That document, Tuttle Exhibit 33, does not include a specific definition of nonasbestos-containing, agreed?
MR. FROST: Objection.
A. Again, as I said, this provides guidelines regarding whether components need to be listed as an ingredient on an MSDS or need to be listed or taken into account when assessing labeling or hazard assessment guidelines and things like that for which they have a .1% cutoff for carcinogens such as asbestos.
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BY MR. SOILEAU:
Q. There's no definition of nonasbestos-containing in Tuttle Exhibit 33, is there, Doctor?
MR. FROST: Objection.
A. Again, as I said, it provides guidelines on whether something is required to be included on an MSDS as an ingredient or a component of a product, and guidelines regarding the labeling and hazard identification.
BY MR. SOILEAU:
Q. Is it your testimony to this court, Doctor, that as you suggest on page 34 of your report, Tuttle Exhibit 33 includes a definition of nonasbestos-containing?
MR. FROST: Objection, misstates the report, misstates her testimony.
A. Again, so as I state in that one sentence and later on again I cite something, the EPA guidelines, which do offer a specific definition for asbestos-containing, which is actually more

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lenient than the Global Harmonization Standard.
What the Global Harmonization Standard does is provide guidelines over what is required to be listed as an ingredient or component in regards to labeling.
MR. SOILEAU: I think I'm just required to move to strike as nonresponsive.
BY MR. SOILEAU:
Q. You didn't cite EPA in that sentence, did you?
MR. FROST: Objection.
A. No, I cite it in the next sentence, I believe.
BY MR. SOILEAU:
Q. Well, you cite many, many articles through your report. I mean, you've got pages and pages of references that you cite through your report, right?
A. Yes, that's true.
Q. But I'm only asking about one sentence. You understand that?
A. Well, again, you're asking

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about an asbestos-containing definition, and in the very next sentence in my report --
Q. No. No, I'm asking you: You say this by definition -- and you used the words "nonasbestos-containing." You open paren, United Nations, comma, 2017, close paren, period. It is the only citation you offer for that sentence.
And I'm asking you if you can at least acknowledge for the record that that document, in its many, many pages, Tuttle Exhibit 33 does not include a definition of nonasbestos-containing.
MR. FROST: Objection.
BY MR. SOILEAU:
Q. That's all I'm asking. It's very straightforward.
A. Well, again, the reason it is the only reference cited in that particular point is we are talking about the .1% cutoff. In the next sentence we go to the 1% cutoff established by US EPA that does actually define asbestos-containing.
What the Global Harmonization

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Standard does, as I said previously, provides guidelines on whether materials have to be included as an ingredient in a material safety data sheet or on a label or a hazard label put on a product.
Q. Is that the best answer you can offer to the question I asked?
MR. FROST: Objection.
A. I'm trying to be as clear as I can be.
BY MR. SOILEAU:
Q. I have to disagree with you, Doctor. I'm asking you if the document in front of you includes a definition of nonasbestos-containing, and it can be no, yes or I don't know.
MR. FROST: Objection, asked and answered.
BY MR. SOILEAU:
Q. But it is the one document --
MR. SOILEAU: It has been asked, but it has not been answered.
MR. FROST: Disagree. Objection.

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MR. SOILEAU: Well, you're stipulating that it's not in there?
MR. FROST: I'm not testifying here today.
MR. SOILEAU: Do you stipulate that she has testified it's not in there?
MR. FROST: I -- the record says what it says.
MR. SOILEAU: Very good. I agree.
MR. FROST: I stipulate that you've asked this question several times and the witness has given answers to it several times.
MR. SOILEAU: I will agree with that.
BY MR. SOILEAU:
Q. It's not in there, is it, Doctor?
MR. FROST: Objection.
A. So --
BY MR. SOILEAU:
Q. You can't say it?

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A. Again, as I said before, I don't believe the word "asbestos-containing" specifically occurs in this document, but that being said, there are guidelines regarding whether the -- and again, it's that .1%, whether a material is required under the Global Harmonization Standard to be listed as an ingredient of a product on a material safety data sheet or on a label.
Q. Let's look at what I have marked as Tuttle Exhibit 34.
(Whereupon, Deposition Exhibit Tuttle-34, IARC Monograph on Asbestos, was marked for identification.)
THE WITNESS: If I may, I'm going to need a short break in the near future.
MR. SOILEAU: We're going to take it as soon as we look at this. I was thinking the same.
THE WITNESS: Okay.
BY MR. SOILEAU:
Q. Do you recognize the document that I have given you as Tuttle Exhibit 34?

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A. Yes, I do.
Q. What is it?
A. I believe it is the IARC monograph for asbestos.
Q. All right.
MR. FROST: Again, I just want to -- for the record, this is the 2012, the 100C? It doesn't have a cover on it.
MR. SOILEAU: I believe that this is 100C, and I'm sorry, I don't see it.
MR. FROST: That's okay. I just wanted to make the record clear because there are two asbestos monographs.
MR. SOILEAU: I agree.
THE WITNESS: That's true. Thank you.
MR. FROST: Well, that's true. There are some older ones.
MR. SOILEAU: I think what we'll do is go ahead and take a break because I have a page reference here and I don't see what I want to ask you about, and I don't want you to sit here while I look for it.
So we'll take our break now and come back and I'll pick up with this document.
THE VIDEOGRAPHER: Going off the record at 4:56 p.m.
(Recess taken, 4:56 p.m. to 5:06 p.m.)
THE VIDEOGRAPHER: We're back on the record at 5:06 p.m.
BY MR. SOILEAU:
Q. Okay. Doctor, I think we've reached our last session for this deposition.
Are you ready to proceed?
A. Yes, I am.
Q. What is fibrous talc?
A. So I'm going to be speaking, you know, without exact definitions in front of me, but it is my general understanding that fibrous talc is an elongated particle that meets the World Health Organization or OSHA definitions in regarding to fiber size.

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Q. Is the question of fibrous talc relevant to the issues that we discussed here today?
MR. FROST: Objection, vague.
A. Generally speaking, you know, in regards to assessing the causal association between talcum powder and ovarian cancer, the testing that we look at in the scientific literature regarding talcum powder insofar as it contains any materials, you know, that would be included in those -- those studies.
BY MR. SOILEAU:
Q. Do the talcum powder products sold by Johnson & Johnson include, in part, fibrous talc?
MR. FROST: Objection, outside of this witness' area of expertise.
BY MR. SOILEAU:
Q. Is that true?
A. Again, I do not -- I think I have one section where I discuss fibrous talc and it's not in relation to its content or lack thereof in Johnson & Johnson baby powder

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products.
As we discussed earlier, I'm not a mineralogist. I'm not a geologist. I do not offer any opinions in regards to that. I believe others get into the mineralogy more than I do.
Q. Do you know whether fibrous talc is carcinogenic?
A. I am not aware that fibrous talc has been classified as a carcinogen.
Q. All right. Did you rely on any of the testing documents that are part of Appendix C, and specifically the asbestos test in those documents?
A. Again, in my report, I do not get into specifically the testing. I don't reference the asbestos testing in those documents in my report. I look at the scientific literature. I do believe I reference the FDA testing of Johnson & Johnson baby powder regarding asbestos.
In my report, I look at Drs. Longo and Rigler, but I don't believe I look at those Imerys and J&J documents as far

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as assessing asbestos content in the Johnson & Johnson's baby powder.
Q. Do you know whether any of the tests were positive for asbestos, that is, the test results included in Appendix C of your report?
MR. FROST: Objection.
A. As I said, I didn't go through them, I didn't assess the methodology. I know that there are others that look at that specifically and look at the methodology and the conclusions of those testings.
BY MR. SOILEAU:
Q. Right. But you oversaw the design and creation of Appendix C. You told me that earlier, right?
A. Yes, that's correct.
Q. Does Appendix C include in those charts any positive findings on asbestos?
MR. FROST: Objection.
BY MR. SOILEAU:
Q. You know, in the testing.
A. As I said, there is asbestos

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testing in the appendix, and -- but I did not summarize it in my report. I don't recall. If they looked at the baby powder specifically. I know that there was asbestos testing done and I think that they did have some detections.
But again, I did not assess the methodology or the results to be able to discuss those. As I understand, there are others who have done that in more detail, and, you know, they have more -- as I said, I'm not a mineralogist and they discuss that in more detail as regards to the methodology.
Q. So are there any positive findings, though, included in those many pages of charts?
MR. FROST: Objection.
A. Again, we can go through each individual chart. I am not aware specifically of any specific to Johnson & Johnson baby powder. As I said, I know there's asbestos testing throughout the table, some of which were positive, but I did not assess them or look at the methodology

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that was used in those, and I believe there are others who do that and -- who do that.
BY MR. SOILEAU:
Q. Okay, Doctor. Let's return now to the IARC monograph that we had before our last break, Tuttle Exhibit 34. Let's see if I can do this this time without losing my place.
So I've turned to page 294 of Tuttle Exhibit 34, and I'm on Section 5, Evaluation. And I'm looking at the second sentence of that section, Evaluation. It says, quote: "Asbestos causes mesothelioma and cancer of the lung, larynx and ovary," closed quote.
Do you see that statement in the IARC monograph?
A. Yes, I see that statement.
Q. Do you agree with that statement?
A. As I said previously, I'm aware that IARC has found that exposure to high occupational levels of asbestos was associated with ovarian cancer.

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Q. Yes, Doctor, but I'm not asking you if IARC said that or if you are aware that IARC said that. I'm asking you, as a toxicologist, an expert witness put forward in this case on behalf of Johnson & Johnson, if you agree that asbestos causes mesothelioma and cancer of the lung, larynx and ovary?

MR. FROST: Objection, form.

A. Well, again, as I said previously, I'm aware that IARC has stated that high occupational exposures to asbestos is associated with ovarian cancer. In this particular -- I was retained to look at the body of evidence regarding talcum powder and ovarian cancer.

BY MR. SOILEAU:

Q. What level of -- well, let me ask you this.

Can we agree that there's no safe level of asbestos for talcum powder products?

MR. FROST: Objection.

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BY MR. SOILEAU:

Q. Do you agree with that statement? There is no safe level of asbestos in the talcum powder products?

MR. FROST: Objection.

BY MR. SOILEAU:

Q. For Johnson & Johnson?

MR. FROST: Still objection.

A. Well, again, you know, we were discussing earlier the Global Harmonization Standard and the EPA guidelines that have definitions regarding whether a material is considered asbestos-containing or not, and then we also briefly discussed fibrous talc, you know, and we haven't discussed other things.

I know there's been some discussion regarding the methodology of, you know, cleavage fragments in elongated particles as opposed to asbestos.

BY MR. SOILEAU:

Q. Wait. You said cleavage. We didn't talk cleavage.

A. You're right, we didn't discuss

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cleavage.

Q. That was in Mr. Moore's deposition.

MR. FROST: Objection.

BY MR. SOILEAU:

Q. Well, we haven't talked about it today.

A. No, you're correct, we haven't discussed --

Q. Have you done any work on that for Johnson & Johnson?

MR. FROST: Objection.

A. No, I have not.

BY MR. SOILEAU:

Q. Okay. All right. Go ahead, I'm sorry.

A. I'm just saying that in regards to my report and the research I've done, I've been looking at the scientific evidence regarding talcum powder and the causal association with ovarian cancer and whether the scientific evidence supports it.

I'm aware that IARC has, as I stated previously, found that high

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occupational exposure levels of asbestos is associated with ovarian cancer, but I have not, you know, gone into the data regarding asbestos, especially occupational levels of exposure. I summarize it briefly in my report.

Q. Does asbestos cause ovarian cancer, Doctor?

MR. FROST: Objection, asked and answered.

MR. SOILEAU: If there was an answer in all of that, I promise you, I didn't hear it and I lost it.

BY MR. SOILEAU:

Q. I mean, do you have an opinion? You're a toxicologist. You're here. We're talking about ovarian cancer. You knew we were going to talk about ovarian cancer, and you knew asbestos is an issue. You did the Appendix C. You've seen Longo and Rigler and you talk about asbestos in your report.

Can asbestos cause ovarian cancer, Doctor?

MR. FROST: Objection, form.

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A. Well, and again, you know, IARC has certainly stated that heavy occupational exposures to asbestos are associated with ovarian cancer.
BY MR. SOILEAU:
Q. Do you believe that's true?
A. I believe that is what IARC has stated. For my -- but for my intents and purposes, I'm looking at talcum powder in ovarian cancer, and as you discussed, we were talking about asbestos testing with talcum powder.
But the scientific evidence regarding talcum powder, if it does contain asbestos, which as I stated previously, there are others who get into that in more detail than I do, all the epidemiological studies and all the testing that's been done on the talcum powder product would include any materials that were included in the product, including -- you know, if -- so if asbestos was shown to be present, it would be included in those studies.
Q. I was asking if it caused -- if

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asbestos caused ovarian cancer, but I tell you what, I give up. I don't think I can get an answer.
Let me ask you this: If it does, how does it get there?
MR. FROST: Objection.
A. Again, I was looking -- I've been looking at talcum powder and ovarian cancer. I'm aware that IARC has classified it. I have not done research into the occupational levels of exposure in ovarian -- in asbestos and ovarian cancer.
BY MR. SOILEAU:
Q. Doesn't that trouble you?
MR. FROST: Objection.
BY MR. SOILEAU:
Q. Do you know how asbestos could cause ovarian cancer?
MR. FROST: Objection.
BY MR. SOILEAU:
Q. However -- if it's occupational, if it's heavy, if it's asbestos workers, if they're cutting asbestos insulation, how does asbestos find its way to

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the ovary?
MR. FROST: Objection. Outside of the opinions being rendered by this witness.
A. So again, I was not asked to look at the causal association between occupational exposures to asbestos and ovarian cancer. I was asked to look at the causal association between talcum powder exposure and ovarian cancer and whether there's scientific evidence to support it.
BY MR. SOILEAU:
Q. Well, you knew that asbestos in the talcum powder products is an issue, don't you?
MR. FROST: Objection.
A. Well, as I said, I am aware that there's been testing. I'm aware the FDA has tested Johnson & Johnson's talcum powder and shown there was no asbestos, and I'm aware that there is other -- there are other experts in this litigation that look at that in greater detail than I have.
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BY MR. SOILEAU:
Q. Then you must know it's an issue in the case.
MR. FROST: Objection.
BY MR. SOILEAU:
Q. Why would we be talking about it if it's not an issue in the case?
MR. FROST: Objection.
A. But again, regarding establishing the scientific literature, if you assume that the Johnson & Johnson talcum powder products or that talcum powder contains asbestos, then all the studies that look at talcum powder in epidemiological studies or in other scenarios, then they would contain the hypothetical asbestos as opposed to -- and would have it -- so, therefore, it's included in that scientific literature.
BY MR. SOILEAU:
Q. I'm not sure if you're not hearing my question or not answering it despite hearing it, but I need to ask again.
If we assume that asbestos can

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cause ovarian cancer, how does it get there?
MR. FROST: Same objections as I lodged before.
A. And again, I did not research that in -- for this particular thing regarding asbestos, so I -- I don't know. I can't speak to it.
BY MR. SOILEAU:
Q. Does it?
MR. FROST: Objection.
A. Again, I didn't research it. I can't speak to it.
BY MR. SOILEAU:
Q. The fact that asbestos is a cause of ovarian cancer, doesn't that tell you as a toxicologist that it's making its way to the ovary?
MR. FROST: Objection.
A. Again, as I said before, I know that IARC has found that heavy occupational exposures are associated with ovarian cancer. I was asked to evaluate the science regarding perineal exposure to talcum powder and ovarian cancer, not the body of science

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regarding occupational exposures to asbestos and ovarian cancer.
BY MR. SOILEAU:
Q. As a toxicologist, 255 hours of looking at this issue, your background, training and experience and the fact that you've worked in asbestos litigation before, doesn't it matter to you if there is asbestos in the talcum powder products?
MR. FROST: Objection. Certainly outside the scope of this witness' testimony she's giving here today on behalf of Johnson & Johnson.
MR. SOILEAU: I don't know why because she offers an opinion about causation. She has asbestos in her report. She offers a causation discussion in her report and she's got an appendix, which is some 60-plus pages, I think, of a chart that includes asbestos testing.
But in any event, subject to the objection.
THE WITNESS: Okay. Can you

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repeat the question for me, please?
MR. SOILEAU: Sure.
BY MR. SOILEAU:
Q. Does it matter to you if the talcum powder products, including the baby powder that you're using with your newborn son, contains asbestos?
MR. FROST: Objections.
MS. WOODS: Objection.
THE WITNESS: I'm sorry?
BY MR. SOILEAU:
Q. Does it matter to you if this product has asbestos in it?
MR. FROST: Objection.
A. Well, again, in looking at the scientific evidence, if we assume that the product has asbestos in it, which again, there has been testing that's shown it does not, and there are others involved that get into the testing and the methodologies in more detail.
But if we assume -- and I even -- I think I state this in my report, but even if we assume that the product is

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asbestos-containing, when we look at the scientific literature regarding talcum powder and ovarian cancer, that would include any hypothetical components that were present in the talcum powder.
BY MR. SOILEAU:
Q. Does that mean it matters to you or it doesn't matter to you?
MR. FROST: Objection.
A. It means that if the talcum powder -- you know, again, if we assume that it does contain asbestos, which again, there has been testing to show consistently that it does not, that all the studies that are assessing the potential causal relationship between perineal talcum powder exposure and ovarian cancer would also include any asbestos that was hypothetically present in the talcum powder.
BY MR. SOILEAU:
Q. So it does matter or it doesn't matter --
MR. FROST: Objection.
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BY MR. SOILEAU:
Q. -- to your analysis and your opinions in this case, Doctor?
MR. FROST: Objection.
A. Again, any -- any of the studies that look at talcum powder -- excuse me.
If we assume that the talcum powder -- that Johnson & Johnson's baby powder or Shower to Shower products contained asbestos, even though there has been testing that showed they do not, even if we assume it is there, then the scientific data regarding talcum powder would include any components that were present in the talcum powder when assessing the potential for a causal association between talcum powder perineally and ovarian cancer.
BY MR. SOILEAU:
Q. Does inhalation of talcum powder products offer a pathway to the ovaries?
MR. FROST: Objection.
A. I don't know specifically.

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BY MR. SOILEAU:
Q. Okay. You discuss animal models of talc exposure and toxicity at pages 17 and 18 of your report, correct?
A. Yes.
Q. You have three paragraphs, just three paragraphs there.
A. Yes, that's correct.
Q. Isn't animal study -- well, first, isn't that an early area of focus for you?
MR. FROST: Objection.
A. I did animal studies during my Ph.D., yes.
BY MR. SOILEAU:
Q. Did you ever study veterinary science?
A. My bachelor's is in veterinary science, yes.
Q. That's what I was referring to. Your work now with CTEH, is it generally emergency response?
A. Yes, I think I stated earlier that the majority of my work with CTEH is

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emergency response and nonlitigation consulting.
Q. Right. Were you involved in the BP project at all?
A. No, I was not.
Q. Are you aware of any controversy involving CTEH's role in the BP spill?
MR. FROST: Objection.
A. Very generally, I'm aware of a news article that I believe CTEH drafted a response to. I don't know any, yeah, real specifics. I wasn't involved. It was before my time with the company.
BY MR. SOILEAU:
Q. In the materials that you presented with your report and your report, I think, in speaking of qualifications, says that CTEH is associated with the University of Arkansas Medical Sciences BioVentures program.
Do you recall that?
A. Yes, I believe that that's stated in my report.

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Q. CTEH is not associated with the University of Arkansas Medical Sciences school, is it?
A. So it's associated with their BioVentures program, and again, this is obviously before my time, so I can only speak in generalities, but the -- it was originally a start-off from professors at the University of Arkansas.
Q. Well, isn't it true that the BioVentures program is a program that generates spinoff companies that are in the same area generally that CTEH is in?
A. I couldn't speak to other companies that went through the BioVentures program. I didn't go to the University of Arkansas, and as I said, that was years before I joined CTEH.
Q. Have you heard the phrase "spinoff companies"?
A. I --
Q. I'm sorry, I apologize. Just to be more clear, in connection with this relationship of CTEH and the BioVentures

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program?
A. Again, I don't know that I have specifically.
Q. All right. Take a look at that document, tell me if you've seen that before. That may be a website printout, by the way, if it helps you.
THE REPORTER: Is that marked?
THE WITNESS: 35.
MR. SOILEAU: Yes, 35.
(Whereupon, Deposition Exhibit Tuttle-35, Listing of BioVentures Spinoff Companies, was marked for identification.)
BY MR. SOILEAU:
Q. Have you seen that before?
A. No, I have not.
Q. All right. Let's go back to the animal studies for a moment. When you wrote your report, you had Dr. Plunkett's report, didn't you, Doctor?
A. Yes, I did.
Q. Do you know whether you considered -- well, did you identify the

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animal studies that Dr. Plunkett cited and discussed in her report? In other words, did you take her report and see what studies she had looked at?
A. I certainly looked at the references cited in Dr. Plunkett's report. I don't recall how in depth I went regarding animal studies as you -- we noted previously. I just briefly touch on animal models of talc exposure and toxicity in section 7.1.
Q. Right. That struck me. Isn't it true that animal studies are sort of the foundation of toxicology?
MR. FROST: Objection.
A. There are a lot of different -- you know, the foundation of toxicology is the dose makes the poison. In vitro animal studies and epidemiological or human studies all are encompassed in the science of toxicology and toxicological research.
BY MR. SOILEAU:
Q. So it's your testimony that toxicologists deal with in vitro, in vivo and epidemiological studies equally?

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MR. FROST: Objection.
BY MR. SOILEAU:
Q. Is that what you're telling me?
A. As I've said, the in vitro, animal, in vivo or human studies all play a role in toxicological research.
Q. Equally?
A. It would depend on the area of toxicology that you're discussing as far as which one would be used or encountered more frequently.
Q. Are you familiar with a study called Shim, S-H-I-M, 2015, that is noted in Dr. Plunkett's report, paragraph 63, page 43?
A. I -- if you could -- you'd have to provide me where she refers to it in Dr. Plunkett's report.
Q. Right. Okay. Well, I'm not going to take the time to do that right now. We've had some long answers and we're going to run out of time shortly.
Can we agree that oxidative stress produced by talc is evidence of toxicity of talc?

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MR. FROST: Objection.
BY MR. SOILEAU:
Q. If we have a study that shows oxidative stress produced by talc, is that evidence of toxicity for talc?
MR. FROST: Objection.
A. It would -- it would depend on the study. As I mentioned earlier, the inflammatory response and things like that are normal processes in the body, and I would need to see the study and what they were looking at and what the conclusions were to -- and what avenue you're discussing regarding mechanism of toxicity.
BY MR. SOILEAU:
Q. How about you look at your reference materials and see if you discuss or include in your reference materials a report and animal study by Shim, S-H-I-M, from 2015.
A. No, I do not.
Q. What about Radic, R-A-D-I-C, a 1988 study cited by Dr. Plunkett at page 39 of her report, paragraph 59, for suppression of immune system function? Do you include

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that in your reference materials?
A. No, I do not.
Q. Is suppression of the immune system function evidence of toxicity?
A. Again, it depends. That's a very general term. There's not one universal mechanism of toxicity.
Q. Well, can we agree that if Dr. Plunkett cited the Radic 1988 study in her report, that you had notice of this report, this study, and an opportunity to look at both Shim and Radic before you rendered your report?
MR. FROST: Objection.
A. Again, my report, my goal was to assess the scientific literature regarding the potential for perineal talc exposure and causal association with ovarian cancer. I read Dr. Plunkett's report. As I said, I looked at the data Dr. Plunkett cites. I don't claim to have cited every reference that Dr. Plunkett cites, and my specific addresses to Dr. Plunkett are listed herein and I only briefly touch on animal models or

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the animal studies regarding talc exposure.
BY MR. SOILEAU:
Q. This is a point that we discussed a bit earlier in your deposition. You said just now you looked at the data Dr. Plunkett cites.
Does that mean that if Dr. Plunkett cited Shim and Radic, that you would have looked at those studies?
MR. FROST: Objection.
A. I -- again, I don't claim -- I think I just said that I don't claim to have pulled every single reference that Dr. Plunkett cites. My address -- you know, my references to Dr. Plunkett's report are in my report and what I address specifically, I did not get into the scientific literature regarding an exhaustive search around animal studies. As I said, we only briefly -- I only briefly touch on talc exposure in animal studies.
BY MR. SOILEAU:
Q. I understand that, but no, no, no, no. I'm not asking you about the

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thousands of studies that are out there.
I'm asking you specifically about studies that Dr. Plunkett had in her report that was provided to you as noted at page 2 of your report, and you said to me, quote, "As I said, I looked at the data Dr. Plunkett cites," period. "I don't claim to have cited every reference that Dr. Plunkett cites." And you continue. Your testimony will speak for itself.
I'm just trying to find out if you did, as you said a moment ago, look at the data that Dr. Plunkett cites, including Shim and Radic.
MR. FROST: Objection.
A. So again, you know, the references that I cite in my report are the references that I rely upon in my report.
BY MR. SOILEAU:
Q. But not necessarily everything you looked at.
A. Well, you know, we discussed that previously.
Q. Right.

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A. In looking at Dr. Plunkett's report, my -- the things that I address in her report are listed in my report. I don't -- and again, I don't get into a detailed discussion of the in vivo studies regarding talc exposure.
There's already a lot of evidence in the epidemiological studies in the human studies that I did not spend a large amount of time in the scientific research looking at the animal studies or the in vitro studies, which are useful as we were talking about earlier, but for causation assessment analyses, you know, the human studies are what primarily -- what I emphasized in my report.
Q. Why not?
MR. FROST: Objection.
BY MR. SOILEAU:
Q. So why not?
MR. FROST: Why not what? I don't understand what the why not is to.
MR. SOILEAU: Well, I know,

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it's buried in those long answers, but she said "I don't get into a detailed discussion of the in vivo studies regarding talc exposure."
BY MR. SOILEAU:
Q. And that's my question: Why not?
You also said, "I did not spend a large amount of time in the scientific research looking at the animal studies."
Why not?
A. Because again, I was asked to assess is the scientific literature about a causal association, and while in vivo studies and in vitro studies are useful for looking at various aspects of toxicology, the epidemiological studies and the studies that I spend -- you know, those are the ones that we use as far as epidemiological and human-based, if they're available, for establishing a causal association or seeing if the data supports a causal association.
Q. Let's do a couple more from Dr. Plunkett's report. Beck 1987, for the

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record, it's at page 39 of Dr. Plunkett's report, paragraph 59. It's cited for macrophage phagocytosis inhibited and elevated enzyme levels.
A. And what was the question?
Q. Did you look at the Beck '87 reference list?
A. Again, I don't cite a Beck article in my report.
Q. Okay. Can you agree that elevated enzyme levels is evidence of toxicity?
MR. FROST: Objection.
A. Again, it would depend, as we discussed previously, there's not a universal mechanism of toxicity. I would need to see the study and see what the parameters are, what they're looking at.
BY MR. SOILEAU:
Q. If, in a human body, the macrophage phagocytosis is inhibited, is that evidence of toxicity?
MR. FROST: Objection.
A. Again, it depends.

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BY MR. SOILEAU:
Q. Are animal studies -- can we take animal -- mammalian animal studies and apply those to humans? That is, if we see a toxic effect in mammalian species, is it fair to expect that we will see those in humans as well?
A. No, you need more information. As I said before, animal studies are certainly useful tools, but animal studies are -- you know, animals do not share identical mechanisms or physiological impacts to humans.
And so you can't just say that if it exerts a toxic effect in an animal that it will assert a toxic effect in a human. You would need more research and more information.
Q. What good are animal studies if we can't apply them to humans?
MR. FROST: Objection.
A. Well, again, what I said was that you need more information as far as animal studies. Animal studies are useful,

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they help us understand different things depending on the questions asked, but you cannot just take a single animal study and directly apply it to human toxicity or human -- human information.
BY MR. SOILEAU:
Q. And you're saying animal. I just want to make sure it's clear that I'm asking you specifically about mammalian studies. Is that understood?
A. Yes.
Q. Okay. But your testimony is the same, right?
A. Well, again, as I said, you know, animal studies are useful. As far as the application to human, you would need more information there. You know, as I stated before, the anatomy and physiology of animals is not identical to human anatomy and physiology.
Q. What animal model is the most appropriate to study ovarian cancer?
MR. FROST: Objection.
///

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BY MR. SOILEAU:
Q. Do you know?
A. I briefly discussed some of the ovarian -- models, animal models of ovarian cancer. It would depend on what question you were wanting to answer.
Q. Well, I'm just -- if I -- if the object is to study ovarian cancer, which model is the most appropriate?
MR. FROST: Objection.
A. Again, it would depend on the hypothesis that's being tested.
BY MR. SOILEAU:
Q. Let me show you what I'm going to mark as Tuttle Exhibit 36.
(Whereupon, Deposition Exhibit Tuttle-36, NTP Study, Toxicology and Carcinogenesis Studies of Talc, was marked for identification.)
BY MR. SOILEAU:
Q. Is this the NTP study, Doctor, that you reference in your report?
A. I'm just double-checking. Yes, I believe it is.

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Q. Very well.
I know you have a number of things to say about this report, but I want to ask you: Isn't it true -- well, first, do you know what an interim evaluation is?
A. I guess it would depend on the context, but --
Q. Well, in the context of an NTP study like this one I just put in front of you.
A. Do they -- I mean an interim evaluation, I guess I'd have to see it used in the context. Like I said, I know what interim and evaluation mean, but as far as if it's a preliminary study or if the results are not finished yet or what they mean when they say interim evaluation.
Q. Well, what about if I say interim sacrifice, does that help?
A. No, that doesn't.
Q. Okay. Turn to page 54 of this exhibit, which I have marked as Tuttle 36.
MR. ZELLERS: I'm sorry, what page, Counselor?

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MR. SOILEAU: It's on page --
MR. FROST: 54.
MR. SOILEAU: -- 54. Thank you.
BY MR. SOILEAU:
Q. I'm interested in the language here that begins within the paragraph to the left below the chart. It says: The interim evaluations in the NTP talc study clearly demonstrate a progressive impairment of homeostatic growth regulation in the areas of chronic inflammation and fibrosis associated with talc deposition in rats.
You're aware of this finding?
A. As I said, I've read this article or, I'm sorry, I've read this research by the NTP. I don't know specifically that it's a finding. Like you say, it's a large document, and there have been several documents that I've --
Q. There have been several --
A. -- read.
Q. Several whats? I didn't hear you.

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A. Oh, I said there have been a lot of articles that I've been reading in accordance with this.
Q. Let's just focus on this sentence then. It's not to be a memory test so much. Do you know first what interim evaluations mean here?
You said context before. You know what interim means, you know what evaluation means, but do you know what is meant by interim evaluations as used here at page 54 of this NTP study?
A. Judging from what it's saying here, it sounds like it's -- you know, and again, I'm not the author of this document, so I'm merely having to base this on a single sentence, again, not -- not the entire context of the document.
But it looks like there -- maybe another word would be preliminary evaluations or evaluations in the ongoing -- the ongoing study.
Q. Okay. And they say that the interim evaluations in the NTP talc study

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clearly demonstrate a progressive impairment of homeostatic growth regulation in the areas of chronic inflammation and fibrosis associated with talc deposition in rats.

What does that mean to you, Doctor, as a toxicologist?

A. Well, again, taking the one statement, you know, out of this very substantial document, and there's about five pages of discussion and conclusions that precede this particular paragraph, there are -- so I don't know that I can speak to what the authors are specifically saying here. There's...

Q. What is homeostatic growth regulation?

A. Again, I don't feel comfortable. I don't think I can define that scientifically without having a reference in front of me. I don't want to misspeak.

Q. Okay. I'm going to the next sentence. It says: Hyperplasia -- hyperplasia of the alveolar epithelium.

Do you know what hyperplasia

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is?

A. Again, yes. As far as a definition, I wouldn't want to misspeak and misquote the scientific definition in the media -- or sorry, not in the media -- in the scientific literature.

Q. How and why, if at all, is a finding of hyperplasia in the epithelium tissue that's evident at six months and that became more extensive and severe with duration of exposure significant to the issues we discuss here today, Doctor?

A. Well, again, it would depend. In this particular study, it's talking about the lung alveolar and inhalational exposures. I would need to look at more information to determine whether it is applied here.

As I said previously, I looked at the perineal application of talcum powder and the causal association with ovarian cancer.

Q. Does hyperplasia have any relationship to carcinogenicity or the development of cancer?

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A. Well, I believe, if you look at the, actually, next sentence, we do have a definition as far as this NTP document regarding hyperplasia, where they say there was an increased number of cells.

Q. What's that mean, Doctor?

A. It would mean there's an increased number of cells in the alveolar regions that they were looking at in this particular study.

Q. What's going on here? What's NTP seeing? Do you know?

A. Hmm?

Q. What is NTP seeing here in these results?

A. NTPC?

Q. NTP. What did I say?

A. You said NTPC.

Q. No. I'm sorry. I meant "see" as with observational.

A. Oh.

Q. Can you help me understand what's going on here in this section of this report from the National Toxicology Program,

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a report that you cited?

A. Again, without being able to refamiliarize myself with the report, just taking a couple of sentences in the discussions and conclusions, I -- I am afraid I can't help you as far as what is NTP, you know, looking at.

As I said before, I've read the report, but I don't have a photographic memory, so I can't remember all the nuances in it and all the context.

Q. Very well, Doctor.

Let's go back to Exhibit 3. That is the Reference Manual on Scientific Evidence. You recall we discussed that earlier? I'm looking at page 646.

Do you see the heading D, Extrapolation from Animal and Cell Research to Humans on page 646 of Tuttle Exhibit 3, the Reference Manual on Scientific Evidence?

A. Yes, I see it.

Q. Do you see the second sentence that says: In qualitative extrapolation, one can usually rely on the fact that a compound

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causing an effect in one mammalian species will cause it in another species, end of quote.

Do you see that?

A. I see that, where it states that, but again, I'd have to look at the rest of the paragraph and the rest of the section.

Q. Sure. Do you agree with that statement, though?

MR. FROST: Objection.

A. Well, as I said before, and this is dealing in qualitative extrapolations --

BY MR. SOILEAU:

Q. It is.

A. As I said previously, we would need more information in regards to an animal study and the extrapolation to humans.

As I said previously, the anatomy and physiology in animals is not identical to that in humans, and there are -- in fact, it says some different things down here about the mathematical depiction for pharmacokinetics and toxicokinetics for

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extrapolating those kinds of studies to humans.

Q. Doctor, isn't it true that this statement that I read stating: In qualitative extrapolation, one can usually rely on the fact that a compound causing an effect in one mammalian species will cause it in another species, is a basic principle of toxicology?

MR. FROST: Objection.

A. Well, again, as I've stated before, it would depend -- and again, this doesn't say an effect in one mammalian species will cause an effect in humans. It just states another species.

BY MR. SOILEAU:

Q. Well, that's certainly true. It also says this is a basic principle of toxicology, doesn't it? Right there?

A. In the next sentence, yes, it states that.

Q. Do you disagree with that?

MR. FROST: Objection.

A. Well, again, as I said, when

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you are -- you know, that animal and cell research is extremely useful to toxicologists, the extrapolation, you need more information than just a general -- if an adverse effect occurs in an animal study, that it will also occur in humans.

BY MR. SOILEAU:

Q. Do you know if -- if the Casarett text on toxicology that we talked about earlier in your deposition says two main principles underlie all descriptive animal toxicity testing; the first is that the effects produced by a compound in laboratory animals when properly qualified are applicable to humans. This premise applies to all of experimental biology and medicine.

Does that sound familiar to you? I don't have -- I didn't bring this as an exhibit. I didn't know this was going to be an issue, but I have a copy of it here.

Do you recognize that language? Two main principles underlie all descriptive animal toxicity testing; the first is that

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effects produced by a compound in laboratory animals when properly qualified are applicable to humans. This premise applies to all of experimental biology and medicine.

A. Well, again, I don't have the textbook in front of me. You have put it on the overhead.

Q. True, but --

A. You do note that it says when properly -- if you'd put it back up there, please.

Q. Sure. You see, I'm just showing it's Chapter 2, Principles of Toxicology, and I just want to show you that it's Chapter 2, Principles of Toxicology, from Casarett, whether you recognize it or not. I'll put it back up there for you. There you go.

A. So as it says, when properly qualified. And as I say -- are applicable to humans. And as I said, when doing animal research, it is very useful to toxicologists, but to apply it to human studies, you -- to apply it to humans, there are -- you can't

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just -- I can't remember what the exact word I used, but animal studies cannot just be directly applied to humans. There is more information that's needed, and as it states here, it needs to be properly qualified.

Q. Isn't this statement in Casarett that I am showing you consistent with the statement from the reference manual that we looked at a moment ago, page 646 of Exhibit 3?

MR. FROST: Objection.

A. Well, again, as I said, the extrapolation of animal and in vitro studies to humans, you know, those studies are useful for toxicologists, but there needs to be more information when you're doing extrapolation because the anatomy and physiology of animals is not identical to that in humans.

BY MR. SOILEAU:

Q. Are you familiar with the anatomy of ovaries in rats?

MR. FROST: Objection.

A. I'm vaguely familiar with the anatomy. It's not something that I have done

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research on.

BY MR. SOILEAU:

Q. You spend, I think, about 13 pages, which I'm calculating at about 20% of the body of your expert report, dedicated to epidemiology.

Does that surprise you or do you realize that that much attention is paid to epidemiology in your report?

A. I would have to count the pages to confirm whether your numbers are accurate, but I certainly -- as I stated previously, the available scientific literature does have epidemiological studies and the actual examination of the potential association between talcum powder use and ovarian cancer in humans, and so that would certainly be where I would -- you know, I spent a lot of time in my scientific research.

Q. Is it true that the cohort studies that you discuss in those pages of your report do not include specific dose information?

A. We'd have to look at each

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individual cohort study that -- or each study that I cite for me to be able to say specifically.

Q. You can't tell me whether the cohort studies include dose information as we sit here?

MR. FROST: Objection.

BY MR. SOILEAU:

Q. The cohort studies discussed in your report, cohort studies on talcum powder products.

A. Well, as I said, we can look at each individual study and my discussion on them. There are some that attempt to establish some dose information and some that do not, but I have to look at each individual study.

Q. By the way, this NTP study, this discussion we were looking at, at page 54, where it says: Hyperplasia was evident at six months and became more extensive and severe with duration of exposure.

Does that sound like

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dose-response to you?

MR. FROST: Objection.

A. Again, it mentions duration of exposure, which is one component of dose.

BY MR. SOILEAU:

Q. Well, it says more than duration of exposure. It says became more extensive and severe with duration of exposure. Here, you can have my page for a moment.

More severe, more extensive with duration. Isn't that dose-response?

MR. FROST: Objection.

A. Well, again, as I said, duration is one component of dose, so --

BY MR. SOILEAU:

Q. Right.

A. -- that's certainly preliminary, but again --

Q. So doesn't that say --

A. -- we need to look at that -- that is one sentence. I'd need to look at the data supporting it, but duration is definitely a component of dose.

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Q. Right. But when we have increased dose and increased biological effects, that is, when the effects, the adverse biological effects increase when the dose increases, isn't that the essence of dose-response?
A. That is the study of dose-response, whether the symptom or potential adverse health effect is associated with the dose of a product.
Q. Right. And NTP in that sentence is describing something that is dose-responsive?
MR. FROST: Objection.
BY MR. SOILEAU:
Q. An adverse biological effect that is dose-responsive?
MR. FROST: Objection.
A. Again, in that particular sentence where they're looking at the hyperplasias from inhalational exposure, they certainly note that the hyperplasias were associated with duration.
///

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BY MR. SOILEAU:
Q. Did the Schildkraut study that you looked at in your report include a dose-response relationship?
A. I would need to --
Q. You need it. I'll give you a copy marked as Tuttle Exhibit 37.
(Whereupon, Deposition Exhibit Tuttle-37, 2016 Schildkraut et al Publication, was marked for identification.)
BY MR. SOILEAU:
Q. This is a study that you reference in your discussion of epidemiology, isn't it?
A. Yes, I believe so. I'm actually looking for the spot where I reference it in my report.
Q. Okay. Do you want to look in your reference list to confirm that you cite it?
A. Certainly. Yes.
Q. Look at page 25.
A. Yes.

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Q. Okay. Didn't the authors in the study marked now as Tuttle Exhibit 37 note a dose-response relationship with the duration of use and number of lifetime applications?
A. Yes, I believe I state that in my report, that the authors noted a dose-response based on duration and number of lifetime applications.
If I may, I'm sorry.
Q. Go ahead. Have at it.
A. I was just looking at my report on page 25, where I also note that the significance disappeared when they were interviewed prior or after 2014, and I'm looking for the table in the actual report as well.
Q. Okay. Was this study adjusted for litigation-based bias?
MR. FROST: Objection.
A. Again --
BY MR. SOILEAU:
Q. Is that what you're talking about?

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A. You know, they noted that there was a difference between pre and post 2014, and I believe that they state something about the media, and I think I quoted heightened awareness of the exposure as a result of two recent class action lawsuits.
Q. But my question, Doctor, is: Did the authors of this study adjust for that effect?
A. As I said, they examined the individuals that were interviewed by whether they were interviewed prior to 2014 or after 2014.
Q. Did you state that the case-control studies were not consistent in your report?
A. I believe that I stated that the scientific evidence -- and I actually can refer to the summary in my report so I can make sure I'm accurate regarding the consistency.
I believe I say there is little to no consistency among the scientific literature between the studies as shown by

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the variation between population and hospital-based studies and between case-control and cohort studies, mode of genetic talc application and other places, circumstances and times.
Q. What was your conclusion about the -- well, you reviewed the case-control studies in your report, right? You list them and discuss them briefly?
A. Yes, that's correct.
Q. And what was the conclusion that you reached? I don't know that I saw that you said based on these studies I conclude that the case-control studies offer some evidence, no evidence or otherwise describe what evidence those offer.
Do you understand what I'm saying?
A. Yes, I think I do. And if you refer --
Q. Did I miss it?
A. If you refer to page --
MR. FROST: Objection.
MR. SOILEAU: Sorry.

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A. If you refer to page 29 of my report, after the summary of the various scientific literature regarding, you know, the epidemiological studies, there's a section called Conclusion and Bradford Hill Criteria.
BY MR. SOILEAU:
Q. Let me show you something I've marked as Tuttle Exhibit 38.
(Whereupon, Deposition Exhibit Tuttle-38, Article, Retire Statistical Significance, was marked for identification.)
BY MR. SOILEAU:
Q. Have you had a chance to see this comment that was recently published in Nature? Do you recognize that journal, Nature?
A. Yes, I know the journal Nature.
Q. Very good.
Do you receive it or does CTEH receive it?
A. I don't receive it. I'm not sure if CTEH receives it at the corporate

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office. As I said previously, I'm based out of my home right now.
Q. Have you seen this article before today?
A. Yes, I believe I have.
Q. And in what circumstances did you come to see this article?
A. I guess I'm trying to think specifically. I'm not entirely sure. As I said, I think have a passing familiarity. I think I remember seeing something about it generally in the Nature journal.
Q. Do you see the subheading on the first page, Pervasive Problem?
A. Yes, I do.
Q. It says in that paragraph: Let's be clear about what must stop: We should never conclude there is no difference or no association just because a p value is larger than a threshold such as 0.05 or, equivalently, because a confidence interval includes zero. Neither should we conclude that two studies conflict because one had a statistically significant result and the

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other did not. These errors waste research efforts and misinform policy decisions. We agree, and call for the entire concept of statistical significance to be abandoned.
That ends the quote.
Are you in a position to agree or disagree with that statement?
MR. FROST: Objection.
A. I'm sorry, you jumped from that paragraph to -- to the next -- when you were reading that, so I don't know where you -- your last sentence came from.
BY MR. SOILEAU:
Q. Oh, I went to the second page. I'm sorry. I turned the page.
A. No, I mean, when you ended that paragraph, the paragraph ends with "these errors," and then the next paragraph says, "For example," so I don't know where you went to get the --
MR. FROST: Yeah, the "we agree" part.
A. -- last sentence.
MR. FROST: I don't know where

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that's coming from.
MR. SOILEAU: Oh, okay. I'm sorry.
BY MR. SOILEAU:
Q. "These errors waste research efforts and misinform policy decisions." I don't know, so I'm sorry.
Would you like me to read it again?
A. Please.
Q. It says: Let's be clear about what must stop: We should never conclude there is no difference or no association just because a p value is larger than a threshold such as 0.05 or, equivalently, because a confidence interval includes zero. Neither should we conclude that the two studies conflict because one had a statistically significant result and the other did not. These efforts waste research efforts and misinform policy decisions.
Are you in a position to agree or disagree with that?
MR. FROST: Objection.

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A. Well, as we said previously on other things, this is -- this is one paragraph taken out of a three-page -- appears to be an opinion piece or a comment piece as listed at the beginning of the document, so I don't see any scientific data or anything like that.
It appears to be an opinion, so as such, I can't -- I mean, I can't agree or disagree.
BY MR. SOILEAU:
Q. Do you have any idea how many epidemiologists or biostatisticians signed on to this opinion piece?
A. I see three authors, and then I see something about 800 signatories. But I have no clue the qualifications or any of that of who the signatories are.
Q. Or the authors for that matter?
A. That's correct.
Q. I want to ask you a couple of questions about heavy metals. You talk about heavy metals in your report. In your opinion, will any heavy metals included in

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the talcum powder products be transported within the lattice of the talc?
A. I think we're getting out of the realm of what I assess in regards to heavy metals in my report. As I previously said, any of the research around talcum powder and talcum powder products, if we assume heavy metals are present, then that scientific evidence would include any products that would be in the talcum powder.
Q. But if the heavy metal is present, it's present in the talcum powder, right?
A. That's what I said. If we, you know, assume that the heavy metals are present in the talcum powder, then any testing or any of the epidemiological studies that look at talcum powder would include heavy metals or other -- anything that was present in the talcum powder.
Q. Okay. Because the effect, any adverse biological effect presented by the heavy metals would be combined with whatever else is going on with the talcum powder

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products, fair?
A. As I said, any study that would look at the talcum powder products would include anything that was present in the talcum powder.
Q. But does the talc have the ability to transport within the lattice of the talc heavy metals?
MR. FROST: Objection.
BY MR. SOILEAU:
Q. Do you know what I mean by lattice?
A. I -- I was about to just answer that I don't know.
Q. Okay. Do you maintain that the use of background limits for ambient air is appropriate when we're concerned about ovarian tissue?
MR. FROST: Objection.
A. Can you --
MR. FROST: As to what? Yeah.
BY MR. SOILEAU:
Q. Well, in your report you talk ambient air, correct? Background levels of

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exposure based on ambient air and standards that apply to ambient air; is that right?
A. I believe I discuss ambient air in regards to background levels of exposure to asbestos.
Q. And you believe that's an appropriate methodology, that is, that you can use those ambient air standards in the instance of ovarian cancer issues?
A. Well, I'm not aware of ambient air standards. I refer to the fact that everybody is exposed to background levels of asbestos in a brief discussion regarding the available information regarding airborne exposures to asbestos as related to talcum powder use, but for the -- again, for the majority of my report, I'm referencing the perineal application and --
Q. Do you disagree or agree that there is no safe level of asbestos to talcum powder products?
MR. FROST: Objection.
A. I'm sorry, can you repeat that one more time for me?

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BY MR. SOILEAU:
Q. I only want you to answer if you can tell me you agree or disagree, because we -- we're out of time almost.
Is there any safe level or can you agree there is no safe level of asbestos when it comes to talcum powder products?
MR. FROST: Objection.
BY MR. SOILEAU:
Q. Are you able to agree or disagree with that?
A. I can't agree or disagree without more discussion.
Q. Okay. I'm going to have to ask you. You said that you made an election to use Johnson baby powder on your newborn.
Do you use it daily on him?
MR. FROST: Objection, outside of the scope of the expert opinions being offered here.
MR. SOILEAU: Well, I disagree.
MR. FROST: You can answer.
BY MR. SOILEAU:
Q. I mean, do you use it every

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day, each diapering?
A. Not necessarily each diapering, but I believe I use it every day.
Q. Are you aware of the corn starch alternative?
MR. FROST: Objection.
A. I'm aware that some powder products use cornstarch as opposed to talc, yes.
BY MR. SOILEAU:
Q. Did you make a decision to use the talc products instead of the cornstarch products?
A. I don't believe that I made a conscious decision one way or the other. I bought Johnson & Johnson baby powder for use on my child, and in the course of curiosity as moving forward with this, I did check the ingredients, and it is the talc-containing one.
Q. But you understand there's a choice between talcum powder products in the baby powder line and cornstarch products?
MR. FROST: Objection.

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A. I was not specifically aware of that, no.
BY MR. SOILEAU:
Q. Do you know which one you're buying?
MR. FROST: Objection, asked and answered.
A. As I just said, I ended up looking at the bottle after purchasing it and saw that it is the talc-containing one.
BY MR. SOILEAU:
Q. Do you intend to continue to use that product, the talcum powder product, for your newborn?
A. Yes, I do.
Q. Have you read the warning on the bottle?
A. Goodness.
MR. FROST: Objection.
A. I'm trying to remember. I'm sure I probably saw it, but I can't remember what it said off the top of my head.
BY MR. SOILEAU:
Q. Do you know anything about the

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proper use of Johnson's baby powder for an infant?
MR. FROST: Objection.
A. I know how I use it. I don't know what you mean by proper use.
BY MR. SOILEAU:
Q. Do you know if there's a warning about inhalation hazards for Johnson's -- Johnson & Johnson's talcum powder baby powder?
MR. FROST: Objection.
A. Again, I remember there being a -- I think there was a warning. I don't remember specifics about what it said.
BY MR. SOILEAU:
Q. Are you aware of any talcum powder products that carry a warning regarding a risk of ovarian cancer?
MR. FROST: Objection.
A. No, I'm not aware of that.
MR. FROST: How are we doing on time? We're done?
MR. SOILEAU: Okay. I'm going to have to note just for the purposes

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of the record, we've had a lot of long, nonresponsive answers, so I think we will reserve our right to consider a request for more time if necessary just because of the nonresponsiveness and length of some of the answers.
MR. FROST: Okay. Obviously --
MR. SOILEAU: I know that's something we don't agree on.
MR. FROST: I was going to say, we disagree on that, but that's fine. You can note it for the record.
MR. SOILEAU: That's fine. All right. I think that concludes the deposition.
MR. FROST: Yeah, I don't have any questions.
THE VIDEOGRAPHER: This marks the end of the deposition. We're going off the record at 6:16 p.m.
(Proceedings recessed at 6:16 p.m.)
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CERTIFICATE

I, SUSAN PERRY MILLER, Registered Diplomate Reporter, Certified Realtime Reporter, Certified Court Reporter and Notary Public, do hereby certify that prior to the commencement of the examination, KELLY TUTTLE, Ph.D., was duly sworn by me to testify to the truth, the whole truth and nothing but the truth.

I DO FURTHER CERTIFY that the foregoing is a verbatim transcript of the testimony as taken stenographically by and before me at the time, place and on the date hereinbefore set forth, to the best of my ability.

I DO FURTHER CERTIFY that pursuant to FRCP Rule 30, signature of the witness was not requested by the witness or other party before the conclusion of the deposition.

I DO FURTHER CERTIFY that I am neither a relative nor employee nor attorney nor counsel of any of the parties to this action, and that I am neither a relative nor employee of such attorney or counsel, and that I am not financially interested in the action.

_____
SUSAN PERRY MILLER, RDR, CRR
Fellow of the Academy of Professional Reporters
NCRA Registered Diplomate Reporter
NCRA Certified Realtime Reporter
Certified Court Reporter
Notary Public in and for the State of Texas
My Commission Expires: 7/9/2020
Dated: April 12, 2019

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INSTRUCTIONS TO WITNESS

Please read your deposition over carefully and make any necessary corrections. You should state the reason in the appropriate space on the errata sheet for any corrections that are made.
After doing so, please sign the errata sheet and date it.
You are signing same subject to the changes you have noted on the errata sheet, which will be attached to your deposition.
It is imperative that you return the original errata sheet to the deposing attorney within thirty (30) days of receipt of the deposition transcript by you. If you fail to do so, the deposition transcript may be deemed to be accurate and may be used in court.

ERRATA

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ACKNOWLEDGMENT OF DEPONENT

I, KELLY TUTTLE, Ph.D., do hereby certify that I have read the foregoing pages and that the same is a correct transcription of the answers given by me to the questions therein propounded, except for the corrections or changes in form or substance, if any, noted in the attached Errata Sheet.

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KELLY TUTTLE, Ph.D. DATE

Subscribed and sworn to before me this
_______ day of ______________, 20 _____.
My commission expires: ______________

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Notary Public

LAWYER'S NOTES

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Exhibit R

Casarett and Doull's TOXICOLOGY

The Basic Science of Poisons

Eighth Edition

editor

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chapter 2

Principles of Toxicology

David L. Eaton and Steven G. Gilbert

INTRODUCTION TO TOXICOLOGY

Toxicology is the study of the adverse effects of chemical or physical agents on living organisms. A *toxicologist* is trained to examine and communicate the nature of those effects on human, animal, and environmental health. Toxicological research examines the cellular, biochemical, and molecular mechanisms of action as well as functional effects such as neurobehavioral and immunological, and assesses the probability of their occurrence. Fundamental to this process is characterizing the relation of exposure (or dose) to the response. *Risk assessment* is the quantitative estimate of the potential effects on human health and environmental significance of various types of chemical exposures (eg, pesticide residues in food, contaminants in drinking water). The variety of potential adverse effects and the diversity of chemicals in the environment make toxicology a broad science, which often demands specialization in one area of toxicology. Our society's dependence on chemicals and the need to assess potential hazards have made toxicologists an increasingly important part of the decision-making processes.

Different Areas of Toxicology

The professional activities of toxicologists fall into 3 main categories: descriptive, mechanistic, and regulatory (Fig. 2-1). Although each has distinctive characteristics, each contributes to the other, and all are vitally important to chemical risk assessment (see Chap. 4).

A *mechanistic toxicologist* is concerned with identifying and understanding the cellular, biochemical, and molecular mechanisms by which chemicals exert toxic effects on living organisms (see Chap. 3 for a detailed discussion of mechanisms of toxicity). The results of mechanistic studies are very important in many areas of applied toxicology. In risk assessment, mechanistic data may be very useful in demonstrating that an adverse outcome (eg, cancer, birth defects) observed in laboratory animals is directly relevant to humans. For example, the relative toxic potential of organophosphorus (OP) insecticides in humans, rodents, and insects can be accurately predicted on the basis of an understanding of common mechanisms (inhibition of acetylcholinesterase) and differences in biotransformation for these insecticides among the different species. Similarly, mechanistic data may be very useful in identifying

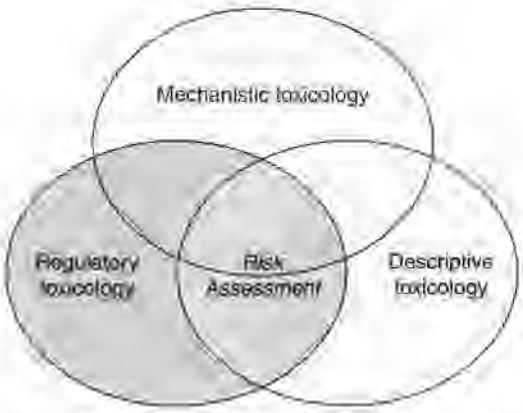

Figure 2-1. Graphical representation of the interconnections between different areas of toxicology.

adverse responses in experimental animals that may not be relevant to humans. For example, the propensity of the widely used artificial sweetener saccharin to cause bladder cancer in rats may not be relevant to humans at normal dietary intake rates. This is because mechanistic studies have demonstrated that bladder cancer is induced only under conditions where saccharin is at such a high concentration in the urine that it forms a crystalline precipitate (Cohen, 1998). Dose–response studies suggest that such high concentrations would not be achieved in the human bladder even after extensive dietary consumption.

Mechanistic data are also useful in the design and production of safer alternative chemicals and in rational therapy for chemical poisoning and treatment of disease. For example, the drug thalidomide was originally marketed in Europe and Australia as a sedative agent for pregnant women. However, it was banned for clinical use in 1962 because of devastating birth defects that occurred if the drug was ingested during a critical period in pregnancy. But mechanistic studies over the past several decades have demonstrated that this drug may have a unique molecular mechanism of action that interferes with the expression of certain genes responsible for blood vessel formation (angiogenesis). With an understanding of this mechanism, thalidomide has been "rediscovered" as a valuable therapeutic agent that may be highly effective in the treatment of certain infectious diseases (eg, leprosy) and multiple myeloma. This provides an interesting example of how a highly toxic drug with selectivity toward a specific population (pregnant women) can be used relatively safely with proper precautions. Following its approval for therapeutic use in 1998, a program was established that required all clinicians, pharmacists, and patients who receive thalidomide to enroll in a specific program (System for Thalidomide Education and Prescribing Safety [STEPS]). The population at risk for the potential teratogenic effects of thalidomide (all women of childbearing age) was required to use 2 forms of birth control and also have a negative pregnancy test within 24 hours of beginning therapy, and periodically thereafter. Among the patients registered with the STEPS program, 6000 were females of childbearing age. Remarkably, after 6 years of use, only 1 patient actually received thalidomide during her pregnancy. She initially tested negative at the beginning of therapy; on a subsequent test she was identified as positive, and the drug was stopped. The pregnancy ended up as a miscarriage (Uhl *et al.*, 2006). Thus, a clear understanding of mechanism of action led to the development of strict prescribing guidelines and patient monitoring, thereby allowing a potentially dangerous drug to be used safely and effectively to treat disease in tens of thousands of patients who would otherwise not have benefited from the therapeutic actions of the drug (Lary *et al.*, 1999).

In addition to aiding directly in the identification, treatment, and prevention of chemical toxicity, an understanding of the mechanisms of toxic action contributes to the knowledge of basic physiology, pharmacology, cell biology, and biochemistry. The advent of new technologies in molecular biology and genomics now provides mechanistic toxicologists with the tools to explore exactly how humans may differ from laboratory animals in their response to toxic substances. These same tools are also being utilized to identify individuals who are genetically susceptible to factors in the environment or respond differently to a chemical exposure. For example, a small percentage of the population genetically lacks the ability to detoxify the chemotherapeutic drug, 6-mercaptopurine, used in the treatment of some forms of leukemia. Young children with leukemia who are homozygous for this genetic trait (about 1 in 300) may experience serious toxic effects from a standard therapeutic dose of this drug (Weinshilboum *et al.*, 1999). Numerous genetic tests for polymorphisms in drug-metabolizing enzymes and transporters are now available that can identify genetically susceptible individuals in advance of pharmacological treatment (Eichelbaum *et al.*, 2006).

The development of new approaches to identifying associations between diseases or adverse outcomes and common genetic variants (polymorphisms) has changed from a focus on individual candidate genes to "genome-wide association studies" (GWAS). GWAS are based on a rapid scan of hundreds of thousands of specific genetic variants (markers called "tag SNP") across the genome of persons affected by a particular disorder or adverse-response phenotype and persons who are not affected, with robust statistical tests to identify associations between a specific genetic marker and the phenotype (eg, disease state or adverse drug response). These tools have resulted in the discovery of many "gene–environment interactions," including associations between adverse drug responses and particular genetic polymorphisms (Wang *et al.*, 2011). Moving from the single, "candidate gene" approach to genome-wide studies has led to the development of the relatively new fields of pharmacogenomics and toxicogenomics. These areas provide an exciting opportunity for mechanistic toxicologists to identify and protect genetically susceptible individuals from harmful environmental exposures, and to customize drug therapies that enhance efficacy and minimize toxicity, based on an individual's genetic makeup.

A *descriptive toxicologist* is concerned directly with toxicity testing, which provides information for safety evaluation and regulatory requirements. The appropriate toxicity tests (as described later in this chapter and other chapters) in cell culture systems or experimental animals are designed to yield information to evaluate risks posed to humans and the environment from exposure to specific chemicals. The concern may be limited to effects on humans, as in the case of drugs and food additives. Toxicologists in the chemical industry, however, must be concerned not only with the risk posed by a company's chemicals (insecticides, herbicides, solvents, etc) to humans but also with potential effects on fish, birds, and plants, as well as other factors that might disturb the balance of the ecosystem. Descriptive toxicology is of course not divorced from mechanistic studies, as such studies provide important clues to a chemical's mechanism of action, and thus contribute to the development of mechanistic toxicology through hypothesis generation. Such studies are also a key component of risk assessments that are used by regulatory toxicologists. The development of so-called omics technologies (genomics, transcriptomics, proteomics, metabonomics/metabolomics, etc) forms the basis of the subdiscipline of toxicogenomics. The application of these technologies to toxicity testing is in many ways "descriptive" in nature, yet affords great mechanistic insights into how chemicals produce their toxic effects. This exciting area of toxicology is discussed in more detail later in the chapter.

A *regulatory toxicologist* has the responsibility for deciding, on the basis of data provided by descriptive and mechanistic toxicologists, whether a drug or other chemical poses a sufficiently low risk (or in the case of drugs, a favorable risk/benefit profile) to be marketed for a stated purpose or subsequent human or environmental exposure resulting from its use. The Food and Drug Administration (FDA) is responsible for allowing drugs, cosmetics, and food additives to be sold in the market according to the Federal Food, Drug and Cosmetic Act (FFDCA). The US Environmental Protection Agency (EPA) is responsible for regulating most other chemicals according to a variety of different legislative acts, including the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA), the Toxic Substances Control Act (TSCA), the Resource Conservation and Recovery Act (RCRA), the Safe Drinking Water Act, and the Clean Air Act. In 1996, the US Congress passed the Food Quality Protection Act (FQPA) that fundamentally changed the pesticide and food safety laws to consider stricter safety standards particularly for infants and children, who were recognized as more susceptible to health effects of pesticides. The EPA is also responsible for enforcing the Comprehensive Environmental Response, Compensation and Liability Act (CERCLA, later revised as the Superfund Amendments Reauthorization Act (SARA)), more commonly called the Superfund Act. This regulation provides direction and financial support for the cleanup of waste sites that contain toxic chemicals that may present a risk to human health or the environment. The Occupational Safety and Health Administration (OSHA) of the Department of Labor was established to ensure that safe and healthful conditions exist in the workplace. The National Institute for Occupational Safety and Health (NIOSH) as part of the Centers for Disease Control and Prevention (CDC) in the Department of Health and Human Services (DHHS) is responsible for conducting research and making recommendations for the prevention of work-related injury and illness. The Consumer Product Safety Commission (CPSC) is responsible for protecting consumers from hazardous household substances, whereas the Department of Transportation (DOT) ensures that materials shipped in interstate commerce are labeled and packaged in a manner consistent with the degree of hazard they present. The Nuclear Regulatory Commission (NRC), established in 1974, regulates the civilian use of nuclear material to protect public health and safety, and the environment. Regulatory toxicologists are also involved in the establishment of standards for the amount of chemicals permitted in ambient air, industrial atmospheres, and drinking water, often integrating scientific information from basic descriptive and mechanistic toxicology studies with the principles and approaches used for risk assessment (see Chap. 4).

In addition to the above categories, there are other specialized areas of toxicology such as forensic, clinical, and environmental toxicology. *Forensic toxicology* is a hybrid of analytic chemistry and fundamental toxicological principles. It is concerned primarily with the medicolegal aspects of the harmful effects of chemicals on humans and animals. The expertise of forensic toxicologists is invoked primarily to aid in establishing the cause of death and determining its circumstances in a post-mortem investigation (see Chap. 31). *Clinical toxicology* designates an area of professional emphasis in the realm of medical science that is concerned with disease caused by or uniquely associated with toxic substances (see Chap. 32). Generally, clinical toxicologists are physicians who receive specialized training in emergency medicine and poison management. Efforts are directed at treating patients poisoned with drugs or other chemicals and at the development of new techniques to treat these intoxications. Public information about treatment and prevention is often provided through the national network of poison control centers. *Environmental toxicology* focuses on the impacts of chemical pollutants in the environment on biological organisms. Although toxicologists concerned with the effects of environmental pollutants on human health fit into this definition, it is most commonly associated with studies on the impacts of chemicals on nonhuman organisms such as fish, birds, terrestrial animals, and plants. *Ecotoxicology* is a specialized area within environmental toxicology that focuses more specifically on the impacts of toxic substances on population dynamics in an ecosystem. The transport, fate, and interactions of chemicals in the environment constitute a critical component of both environmental toxicology and ecotoxicology.

Toxicology and Society

Information from the toxicological sciences, gained by experience or research, has a growing influence on our personal lives as well as on human and environmental health across the globe. Knowledge about the toxicological effects of a compound affects consumer products, drugs, manufacturing processes, waste cleanup, regulatory action, civil disputes, and broad policy decisions. The expanding influence of toxicology on societal issues is accompanied by the responsibility to be increasingly sensitive to the ethical, legal, and social implications of toxicological research and testing.

The convergence of multiple elements has highlighted the evolving ethical dynamics of toxicology. First, experience and new discoveries in the biological sciences have emphasized our interconnectedness with nature and the need for well-articulated visions of human, animal, and environmental health. One vision is that we have "condition(s) that ensure that all living things have the best opportunity to reach and maintain their full genetic potential" (Gilbert, 2001a). Second, we have experience with the health consequences of exposure to such things as lead, asbestos, and tobacco, along with the detailed mechanistic research to understand the long-term risks to individuals and society. This has precipitated many regulatory and legal actions and public policy decisions, not to mention costly and time-consuming lawsuits. Third, we have an increasingly well-defined framework for discussing our social and ethical responsibilities. There is growing recognition that ethics play a crucial role in public health decision making that involves conflicts between individual, corporate, and social justice goals (Callahan and Jennings, 2002; Kass, 2001; Lee, 2002). Fourth is the appreciation that all research involving humans or animals must be conducted in a responsible and ethical manner. Fifth is managing both the uncertainty and biological variability inherent in the biological sciences. Decision making often includes making judgments with limited or uncertain information, which often includes an overlay of individual values and ethics. Finally, individuals involved in toxicological research must be aware of and accountable to their own individual biases and possible conflicts of interest and adhere to the highest ethical standards of the profession (Manrissen *et al.*, 2005; Coble *et al.*, 2009; Gilbert and Eaton, 2009).

Ethical reasoning and philosophy has a long and deep history, but more pragmatic bioethical reasoning can be traced to Leopold, who is arguably America's first bioethicist: "A thing is right when it tends to preserve the integrity, stability, and beauty of the biotic community. It is wrong when it tends otherwise" (Leopold, 1949). The essence of toxicology is to understand the effects of chemicals on the biotic community. This broader definition of an ethic became more focused with examples such as the mercury poisoning in Minamata Bay, Japan, thalidomide, and the effects of pesticides as brought to public awareness by Carson's *Silent Spring* (Carson, 1962). In the United States, these events supported the public and

political will to establish the EPA and strengthen the FDA and other regulations designed to protect human and environmental health. The knowledge that some segments of our society were deferentially at risk from chemical exposures evolved into an appreciation of environmental justice (Corburn, 2002; EPA, 2005; Lee, 2002; Morello-Frosch *et al.*, 2002). The EPA defines environmental justice as "the fair treatment and meaningful involvement of all people regardless of race, color, national origin, or income with respect to the development, implementation, and enforcement of environmental laws, regulations, and policies..." (EPA, 2005). Environmental justice is now an important component of numerous community-based programs of interest, and is relevant to the field of toxicology (Nweke, 2011). There is growing recognition of the direct financial and indirect costs to individuals and society from environmental exposures that are not equally distributed across society (Landrigan *et al.*, 2002).

On a parallel track, biomedical ethics developed out of the lessons of World War II and related abuses of human subjects. The 4 principle of biomedical ethics—respect for autonomy, beneficence (do good), nonmaleficence (do no harm), and justice (be fair)—became well established as a basis for decision making in health care settings (Beauchamp and Childress, 1994). These principles formed the basis of rules and regulations regarding the conduct of human research. The demands of ethics and science made it clear that the highest standards of care produced the best results in both human and animal research. Rules and regulations regarding the housing and conduct of animal studies evolved similarly. Professional toxicology societies now require their members to adhere to the highest ethical standards when conducting research with humans or animals. A further refinement and expansion of biomedical ethical principles is the development of community-based participatory research that takes into consideration community needs to ensure the best results and benefit to the community (Arcury *et al.*, 2001; Gilbert, 2006; O'Fallon and Dearry, 2002).

A glance at the daily newspaper confirms the number of current, sometimes controversial issues that are relevant to the field of toxicology. Decisions and action are often demanded or required even when there is a certain level of uncertainty in the toxicological data. The classic example of this challenge is establishing causation of the health effects of tobacco products. In part to address issues related to the health effects of tobacco products, Hill, a distinguished epidemiologist, defined a set of guidelines for evaluating "causation"—for example, whether a causal connection between a particular "exposure" and a particular outcome, condition, or disease can be scientifically established (Hill, 1965). These criteria are briefly summarized as follows:

1. Strength of association (relationship between independent and dependent variables)
2. Consistency of findings (replication of results by different studies)
3. Biological gradient (strength of the dose–response relationship)
4. Temporal sequence ("cause" before effect)
5. Biological or theoretical plausibility (mechanism of action)
6. Coherence with established knowledge (no competing hypotheses)
7. Specificity of association (cause is tightly linked to an outcome)

Although the guidelines provided by Hill were originally designed for interpretation of epidemiological data, they are equally applicable to establishing causation in toxicology, which often relies on a mix of both epidemiological and toxicological data.

Quantitative risk assessment was developed in part to address issues of uncertainty related to potential harm. The risk assessment process summarizes data for risk managers and other decision makers, who must take into consideration to some degree the qualitative elements of ethical, social, and political issues. Whereas risk management clearly has an ethical and values-based aspect, risk assessment is not immune from the influence of one's values, bias, or perspective. Ultimately action is required and as Hill (1965) noted: "All scientific work is incomplete—whether it be observational or experimental. All scientific work is liable to be upset or modified by advancing knowledge. That does not confer upon us a freedom to ignore the knowledge we already have or postpone the action that it appears to demand at a given time." These so-called Bradford Hill criteria were developed largely as a "weight-of-evidence" approach for interpreting a body of epidemiology data, yet are relevant as well to toxicology. Guzelian *et al.* (2005) provided a more detailed, evidence-based approach for determining causation in toxicology, primarily for application in the legal arena.

Although the scientific data may be the same, there are substantial differences in how toxicological data are used in a regulatory framework to protect public health versus establishing individual causation in the courtroom (Eaton, 2003). The approach to regulatory decision making is in part directed by policy. For example, the experience with thalidomide and other drugs motivated the US Congress to give the FDA broad power to ensure the efficacy and safety of new medicines or medical procedures. In this situation the pharmaceutical company or proponents of an activity must invest in the appropriate animal and human studies to demonstrate safety of the product. In general, a relatively precautionary approach has historically been taken with regard to drugs and medical devices. The approach to industrial chemicals is defined by the Toxic Substance Control Act and does not stipulate such a rigorous approach when introducing a new chemical into commerce.

Building on the work of Hill and others particularly from Europe, the Precautionary Principle was defined at the Wingspread Conference, in 1998: "When an activity raises threats of harm to human health or the environment, precautionary measures should be taken even if some cause and effect relationships are not fully established scientifically" (Gilbert, 2005b; Myers and Raffensperger, 2006; Raffensperger and Tickner, 1999). The precautionary principle incorporates elements of science and ethical philosophy into a single statement, acknowledging that ethics and values are part of the decision-making process. Although the conceptual value of the precautionary principle to public health protection is obvious, its actual implementation in toxicological risk assessment is not straightforward, and remains a point of considerable debate (Marchant, 2003; Goldstein, 2006; Peterson, 2006). The challenge remains to develop a regulatory environment that is responsive to issues of public health and the stewardship of societal resources (Simon, 2011).

With the increased relevance of toxicological data and evaluation in issues fundamental to society, there has been increased awareness of the possibility of conflicts of interest influencing the decision-making process (Maurissen *et al.*, 2005). The disclosure of conflicts of interest as well as the development of appropriate guidelines continues to be a challenge (NAS, 2003; Goozner, 2004; Krimsky and Rothenberg, 2001). These issues go to the core of one's individual values and integrity in the interpretation and communication of research results. Many professional societies, including the Society of Toxicology (http://www.toxicology.org/ai/asot/ethics.asp), have developed codes of ethics for their members. Conflict of interest has also been addressed by most publishers of toxicology journals (Krimsky and Sweet, 2009).

Table 2-1

Approximate Acute LD_{50}s of Some Representative Chemical Agents

AGENT	LD_{50} (mg/kg)*
Ethyl alcohol	10,000
Sodium chloride	4000
Ferrous sulfate	1500
Morphine sulfate	900
Phenobarbital sodium	150
Picrotoxin	5
Strychnine sulfate	2
Nicotine	1
D-Tubocurarine	0.5
Hemicholinium-3	0.2
Tetrodotoxin	0.10
Dioxin (TCDD)	0.001
Botulinum toxin	0.00001

LD_{50} is the dosage (mg/kg body weight) causing death in 50% of exposed animals.

As the field of toxicology has matured and its influence on societal issues has increased, so has the need for the profession to make a commitment to examine the ethical, legal, and social implications of research and practice of toxicology.

General Characteristics of the Toxic Response

One could define a poison as any agent capable of producing a deleterious response in a biological system, seriously injuring function or producing death. This is not, however, a useful working definition for the very simple reason that virtually every known chemical has the potential to produce injury or death if it is present in a sufficient amount. Paracelsus (1493–1541), a Swiss/German/Austrian physician, scientist, and philosopher, phrased this well when he noted, "What is there that is not poison? All things are poison and nothing [is] without poison. Solely the dose determines that a thing is not a poison."

Among chemicals there is a wide spectrum of doses needed to produce deleterious effects, serious injury, or death. This is demonstrated in Table 2-1, which shows the dosage of chemicals needed to produce death in 50% of treated animals (lethal dose 50 [LD_{50}]). Some chemicals produce death in microgram doses and are commonly thought of as being extremely poisonous. Other chemicals may be relatively harmless after doses in excess of several grams. It should be noted, however, that measures of acute lethality such as LD_{50} do not accurately reflect the full spectrum of toxicity, or hazard, associated with exposure to a chemical. For example, some chemicals with low acute toxicity may have carcinogenic, teratogenic, or neurobehavioral effects at doses that produce no evidence of acute toxicity. In addition, there is growing recognition that genetic factors can account for individual susceptibility to a range of responses. Finally, it should be recognized that, for a given chemical, multiple different effects can occur in a given organism, each with its own "dose–response relationship." In some circumstances, effects that occur at low doses may not be evident at higher doses because other adverse responses overwhelm or mask more subtle effects that may occur at low doses. Although some have argued that such low-dose effects, not seen at higher doses, make the classical interpretation of the "dose–response" relationship no longer relevant, such low-dose effects also follow their own "dose–response" relationship, but with a "saturation" of the effect occurring at higher doses that induces other molecular, biochemical, and cellular effects that tend to obscure the effects seen at lower doses. The effects of exogenous chemicals that bind to and activate or inhibit endogenous hormone receptors (so-called endocrine disruptors—see Chap. 21) may often have "low-dose" effects that are quite different from those seen at much higher doses.

CLASSIFICATION OF TOXIC AGENTS

Toxic agents are classified in a variety of ways, depending on the interests and needs of the classifier. In this textbook, for example, toxic agents are discussed in terms of their target organs (liver, kidney, hematopoietic system, etc), use (pesticide, solvent, food additive, etc), source (animal and plant toxins), and effects (cancer, mutation, liver injury, etc). The term *toxin* generally refers to toxic substances that are produced by biological systems such as plants, animals, fungi, or bacteria. The term *toxicant* is used in speaking of toxic substances that are produced by or are a by-product of anthropogenic (human-made) activities. Thus, zearalenone, produced by a mold, is a toxin, whereas "dioxin" (2,3,7,8-tetrachlorodibenzo-*p*-dioxin [TCDD]), produced during the production and/or combustion of certain chlorinated organic chemicals, is a toxicant. Some toxicants can be produced by both natural and anthropogenic activities. For example, polyaromatic hydrocarbons are produced by the combustion of organic matter, which may occur both through natural processes (eg, forest fires) and through anthropogenic activities (eg, combustion of coal for energy production; cigarette smoking). Arsenic, a toxic metalloid, may occur as a natural contaminant of groundwater or may contaminate groundwater secondary to industrial activities. Generally, such toxic substances are referred to as toxicants, rather than toxins, because, although they are naturally produced, they are not produced by biological systems. Distinguishing a "toxin" from a "toxicant" is not always easy. For example, many pesticides, such as the pyrethroids, are synthetic analogs of natural products, such that one would call the pyrethrum found in the chrysanthemum flower a "toxin," but the synthetic (and slightly altered in structure) form produced for use in pesticide formulations would be a "toxicant." Thus, although technically incorrect, many physicians and others involved in the diagnosis and treatment of poisonings often use the term "toxin" to refer to any toxic substance, regardless of origin.

Toxic agents may also be classified in terms of their physical state (gas, dust, liquid, size, eg, nanotoxicology), their chemical stability or reactivity (explosive, flammable, oxidizer), general chemical structure (aromatic amine, halogenated hydrocarbon, etc), or poisoning potential (extremely toxic, very toxic, slightly toxic, etc). Classification of toxic agents on the basis of their biochemical mechanisms of action (eg, alkylating agent, cholinesterase inhibitor, endocrine disruptor) is usually more informative than classification by general terms such as irritants and corrosives. But more general classifications such as air pollutants, occupation-related agents, and acute and chronic poisons can provide a useful focus on a specific problem. It is evident from this discussion that no single classification is applicable to the entire spectrum of toxic agents and that a combination of classification systems or a classification based on

other factors is generally needed to provide the best characterization of a toxic substance. Nevertheless, classification systems that take into consideration both the chemical and the biological properties of an agent and the exposure characteristics are most likely to be useful for regulatory or control purposes and for toxicology in general.

SPECTRUM OF UNDESIRED EFFECTS

The spectrum of undesired effects of chemicals is often broad. Some effects are deleterious and others are not. In therapeutics, for example, each drug produces a number of effects, but usually only one effect is associated with the primary objective of the therapy; all the other effects are referred to as *undesirable* or *side effects* of that drug for that therapeutic indication. However, some of these side effects may be desired for another therapeutic indication. For example, the "first-generation" antihistamine diphenhydramine (Benadryl) is effective in reducing histamine responses associated with allergies, but it readily enters the brain and causes mild central nervous system (CNS) depression (drowsiness, delayed reaction time). With the advent of selective histamine receptor antagonists that do not cross the blood–brain barrier and thus do not have this CNS-depressant side effect, diphenhydramine is used less commonly today as an antihistamine. However, it is widely used as an "over-the-counter" sleep remedy, often in combination with analgesics (eg, Tylenol PM, Excedrin PM), taking advantage of the CNS-depressant effects. Some side effects of drugs are never desirable and are always deleterious to the well-being of humans. These are referred to as the *adverse*, *deleterious*, or *toxic* effects of the drug.

Allergic Reactions

Chemical allergy is an immunologically mediated adverse reaction to a chemical resulting from previous sensitization to that chemical or to a structurally similar one. The term *hypersensitivity* is most often used to describe this allergic state, but *allergic reaction* and *sensitization reaction* are also used to describe this situation when preexposure of the chemical is required to produce the toxic effect (see Chap. 12). Once sensitization has occurred, allergic reactions may result from exposure to relatively very low doses of chemicals; therefore, population-based dose–response curves for allergic reactions have seldom been obtained. Because of this omission, some people assumed that allergic reactions are not dose-related. Thus, they do not consider the allergic reaction to be a true toxic response. However, for a given allergic individual, allergic reactions are dose-related. For example, it is well known that the allergic response to pollen in sensitized individuals is related to the concentration of pollen in the air. In addition, because the allergic response is an undesirable, adverse, deleterious effect, it obviously is also a toxic response. Sensitization reactions are sometimes very severe and may be fatal.

Most chemicals and their metabolic products are not sufficiently large to be recognized by the immune system as a foreign substance and thus must first combine with an endogenous protein to form an antigen (or immunogen). A molecule that must combine with an endogenous protein to elicit an allergic reaction is called a *hapten*. The hapten–protein complex (antigen) is then capable of eliciting the formation of antibodies, and usually at least one or two weeks is required for the synthesis of significant amounts of antibodies. Subsequent exposure to the chemical results in an antigen–antibody interaction, which provokes the typical manifestations of allergy. The manifestations of allergy are numerous. They may involve various organ systems and range in severity from minor skin disturbance to fatal anaphylactic shock. The pattern of allergic response differs in various species. In humans, involvement of the skin (eg, dermatitis, urticaria, and itching) and involvement of the eyes (eg, conjunctivitis) are most common, whereas in guinea pigs, bronchiolar constriction leading to asphyxia is the most common. However, chemically induced asthma (characterized by bronchiolar constriction) certainly does occur in some humans, and the incidence of allergic asthma has increased substantially in recent years. Hypersensitivity reactions are discussed in more detail in Chap. 12.

Idiosyncratic Reactions

Chemical idiosyncrasy refers to a genetically determined abnormal reactivity to a chemical (Goldstein *et al.*, 1974; Levine, 1978; Uetrecht, 2007). The response observed is usually qualitatively similar to that observed in all individuals but may take the form of extreme sensitivity to low doses or extreme insensitivity to high doses of the chemical. However, while some people use the term *idiosyncratic* as a catchall to refer to all reactions that occur with low frequency, it should not be used in that manner (Goldstein *et al.*, 1974). A classic example of an idiosyncratic reaction is provided by patients who exhibit prolonged muscular relaxation and apnea (inability to breathe) lasting several hours after a standard dose of succinylcholine. Succinylcholine usually produces skeletal muscle relaxation of only short duration because of its very rapid metabolic degradation by an enzyme that is present normally in the bloodstream called plasma butyrylcholinesterase (also referred to as pseudocholinesterase). Patients exhibiting this idiosyncratic reaction have a genetic polymorphism in the gene for the enzyme butyrylcholinesterase, which results in a protein that is less active in breaking down succinylcholine. Family pedigree and molecular genetic analyses have demonstrated that the presence of low plasma butyrylcholinesterase activity is due to the presence of one or more single-nucleotide polymorphisms (SNPs) in this gene (Bartels *et al.*, 1992). Similarly, there is a group of people who are abnormally sensitive to nitrites and certain other chemicals that have in common the ability to oxidize the iron in hemoglobin to produce *methemoglobin*, which is incapable of carrying oxygen to the tissues. The unusual phenotype is inherited as an autosomal recessive trait and is characterized by a deficiency in NADH-cytochrome b_5 reductase activity. The genetic basis for this idiosyncratic response has been identified as a single nucleotide change in codon 127, which results in replacement of serine with proline (Kobayashi *et al.*, 1990). The consequence of this genetic deficiency is that these individuals may suffer from a serious lack of oxygen delivery to tissues after exposure to doses of methemoglobin-producing chemicals that would be harmless to individuals with normal NADH-cytochrome b_5 reductase activity.

It is now recognized that many of the so-called idiosyncratic adverse drug reactions and many drug–drug interactions are due to specific genetic polymorphisms in drug-metabolizing enzymes, transporters, or receptors. As discussed previously, the growing field of pharmacogenomics and toxicogenomics has helped to identify the molecular basis for many previously described idiosyncratic responses to drugs and other toxic substances (Wang *et al.*, 2011). However, not all "idiosyncratic" responses to toxic substances are easily described by a single genetic polymorphism in a drug-metabolizing enzyme. It is generally thought that most, but not all, idiosyncratic drug responses are due to a combination of individual differences in the ability to: (1) form a reactive intermediate (usually through oxidation to an electrophilic intermediate), (2) detoxify

that reactive intermediate (usually through hydrolysis or conjugation), and/or (3) exhibit differences in immune response to adducted proteins (Uetrecht, 2007). The role of the immune system in mediating rare drug-induced toxic reactions in the liver, skin, and other organ systems is widely recognized, and specific genetic variants in certain parts of the genome that code for the major histocompatibility complexes (MHCs) give rise to specific immune responses to proteins that have been damaged by reactive intermediates of certain drugs. Thus, it is only the individuals who genetically form sufficient amounts of a reactive drug metabolite, *and* who then have an immune response to the modified protein, who have an adverse response to the drug (Uetrecht, 2007).

For example, troglitazone, introduced into the marketplace in 1997 as an effective treatment for type II diabetes, was subsequently withdrawn from the market because of a relatively rare (1 adverse response per 30,000 patients) but often fatal hepatotoxic response. Subsequent studies of tissues from patients who had developed hepatotoxic responses at the normal therapeutic doses revealed that individuals who lacked functional genes for 2 forms of glutathione *S*-transferase (GSTM1 and GSTT1) were more than 3 times as likely to develop troglitazone-induced hepatotoxicity than individuals with 1 or more functional GSTM1 or T1 genes (Ikeda, 2011). However, this does not explain the rarity of the adverse response, since there were many individuals who lacked GSTM1 and T1 genes who took troglitazone with no evident hepatotoxicity. Further studies have suggested that the idiosyncratic hepatotoxicity from troglitazone also has an immune system component, and genetic differences in specific human lymphocyte antigen (HLA) loci might contribute to idiosyncratic drug-induced hepatotoxicity (Ikeda, 2011).

Immediate versus Delayed Toxicity

Immediate toxic effects can be defined as those that occur or develop rapidly after a single administration of a substance, whereas delayed toxic effects are those that occur after the lapse of some time. Carcinogenic effects of chemicals usually have a long latency period, often 20 to 30 years after the initial exposure, before tumors are observed in humans. For example, daughters of mothers who took diethylstilbestrol (DES) during pregnancy have a greatly increased risk of developing vaginal cancer, in young adulthood, approximately 20 to 30 years after their in utero exposure to DES (Hatch *et al.*, 1998). Also, delayed neurotoxicity is observed after exposure to some OP insecticides that act by covalent modification of an enzyme referred to as *neuropathy target esterase* (NTE), a neuronal protein with serine esterase activity (Glynn *et al.*, 1999). Binding of certain OP chemicals to this protein initiates degeneration of long axons in the peripheral and CNS. The most notorious of the compounds that produce this type of neurotoxic effect is triorthocresylphosphate (TOCP). The effect is not observed until at least several days after exposure to the toxic compound. In contrast, most substances produce immediate toxic effects but do not produce delayed effects.

Reversible versus Irreversible Toxic Effects

Some toxic effects of chemicals are reversible, and others are irreversible. If a chemical produces pathological injury to a tissue, the ability of that tissue to regenerate largely determines whether the effect is reversible or irreversible. Thus, for a tissue such as liver, which has a high ability to regenerate, most injuries are reversible, whereas injury to the CNS is largely irreversible because differentiated cells of the CNS cannot divide and be replaced (although recovery from chemically induced damage to the CNS can occur, primarily through the "plasticity" of the brain that allows developed neurons to learn new functions; see Chap. 16). Carcinogenic and teratogenic effects of chemicals, once they occur, are usually considered irreversible toxic effects.

Local versus Systemic Toxicity

Another distinction between types of effects is made on the basis of the general site of action. Local effects are those that occur at the site of first contact between the biological system and the toxicant. Such effects are produced by the ingestion of caustic substances or the inhalation of irritant materials. For example, chlorine gas reacts with lung tissue at the site of contact, causing damage and swelling of the tissue, with possibly fatal consequences, even though very little of the chemical is absorbed into the bloodstream. The alternative to local effects is systemic effects. Systemic effects require absorption and distribution of a toxicant from its entry point to a distant site at which deleterious effects are produced. Most substances except highly reactive materials produce systemic effects. For some materials, both effects can be demonstrated. For example, tetraethyl lead produces effects on skin at the site of absorption and then is transported systemically to produce its typical effects on the CNS and other organs. If the local effect is marked, there may also be indirect systemic effects. For example, kidney damage after a severe acid burn is an indirect systemic effect because the toxicant does not reach the kidney.

Most chemicals that produce systemic toxicity do not cause a similar degree of toxicity in all organs; instead, they usually elicit their major toxicity in only 1 or 2 organs. These sites are referred to as the *target organs* of toxicity of a particular chemical. The target organ of toxicity is often not the site of the highest concentration of the chemical. For example, lead is concentrated in bone, but its toxicity is due to its effects in soft tissues, particularly the brain. DDT is concentrated in adipose tissue but produces no known toxic effects in that tissue.

The target organ of toxicity most frequently involved in systemic toxicity is the CNS (brain and spinal cord). Even with many compounds having a prominent effect elsewhere, damage to the CNS can be demonstrated by the use of appropriate and sensitive methods. Next in order of frequency of involvement in systemic toxicity are the circulatory system; the blood and hematopoietic system; visceral organs such as the liver, kidney, and lung; and the skin. Muscle and bone are least often the target tissues for systemic effects. With substances that have a predominantly local effect, the frequency with which tissues react depends largely on the portal of entry (skin, gastrointestinal tract, or respiratory tract).

Interaction of Chemicals

Because of the large number of different chemicals an individual may come in contact with at any given time (workplace, drugs, diet, hobbies, etc), it is necessary, in assessing the spectrum of responses, to consider how different chemicals may interact with each other. Interactions can occur in a variety of ways. Chemical interactions are known to occur by a number of mechanisms, such as alterations in absorption, protein binding, and the biotransformation and excretion of 1 or both of the interacting toxicants. In addition to these modes of interaction, the response of the organism to combinations of toxicants may be increased or decreased because of toxicological responses at the site of action.

The effects of 2 chemicals given simultaneously produce a response that may simply be additive of their individual responses or may be greater or less than that expected by addition of their individual responses. The study of these interactions often leads to a

better understanding of the mechanism of toxicity of the chemicals involved. A number of terms have been used to describe pharmacological and toxicological interactions. An *additive* effect occurs when the combined effect of 2 chemicals is equal to the sum of the effects of each agent given alone (eg, 2 + 3 = 5). The effect most commonly observed when 2 chemicals are given together is an additive effect. For example, when 2 OP insecticides are given together, the cholinesterase inhibition is usually additive. A *synergistic* effect occurs when the combined effects of 2 chemicals are much greater than the sum of the effects of each agent given alone (eg, 2 + 2 = 20). For example, both carbon tetrachloride and ethanol are hepatotoxic compounds, but together they produce much more liver injury than the mathematical sum of their individual effects on liver at a given dose would suggest. *Potentiation* occurs when 1 substance does not have a toxic effect on a certain organ or system but when added to another chemical makes that chemical much more toxic (eg, 0 + 2 = 10). Isopropanol, for example, is not hepatotoxic, but when it is administered in addition to carbon tetrachloride, the hepatotoxicity of carbon tetrachloride is much greater than when it is given alone. *Antagonism* occurs when 2 chemicals administered together interfere with each other's actions or 1 interferes with the action of the other (eg, 4 + 6 = 8; 4 + (–4) = 0; 4 + 0 = 1). Antagonistic effects of chemicals are often very desirable in toxicology and are the basis of many antidotes. There are 4 major types of antagonism: functional, chemical, dispositional, and receptor. *Functional antagonism* occurs when 2 chemicals counterbalance each other by producing opposite effects on the same physiological function. For example, advantage is taken of this principle in that the blood pressure can markedly fall during severe barbiturate intoxication, which can be effectively antagonized by the intravenous administration of a vasopressor agent such as norepinephrine or metaraminol. Similarly, many chemicals, when given at toxic dose (TD) levels, produce convulsions, and the convulsions often can be controlled by giving anticonvulsants such as the benzodiazepines (eg, diazepam). *Chemical antagonism or inactivation* is simply a chemical reaction between 2 compounds that produces a less toxic product. For example, 2,3-dimercaptosuccinic acid (DMSA; Succimer) chelates with metal ions such as arsenic, mercury, and lead and decreases their toxicity. The use of antitoxins in the treatment of various animal toxins is also an example of chemical antagonism. The use of the strongly basic low-molecular-weight protein protamine sulfate to form a stable complex with heparin, which abolishes its anticoagulant activity, is another example. *Dispositional antagonism* occurs when the disposition—that is, the absorption, distribution, biotransformation, or excretion of a chemical—is altered so that the concentration and/or duration of the chemical at the target organ are diminished. Thus, the prevention of absorption of a toxicant by ipecac or charcoal and the increased excretion of a chemical by administration of an osmotic diuretic or alteration of the pH of the urine are examples of dispositional antagonism. If the parent compound is responsible for the toxicity of the chemical (such as the anticoagulant warfarin) and its metabolic breakdown products are less toxic than the parent compound, increasing the compound's biotransformation (metabolism) by administering a drug that increases the activity of the metabolizing enzymes (eg, a "microsomal enzyme inducer" such as phenobarbital) will decrease its toxicity. However, if the chemical's toxicity is largely due to a metabolic product (as in the case of the organophosphate insecticide parathion), inhibiting its biotransformation by an inhibitor of microsomal enzyme activity (SKF-525A or piperonyl butoxide) will decrease its toxicity. *Receptor antagonism* occurs when 2 chemicals that bind to the same receptor produce less of an effect when given together than the addition of their separate effects (eg, 4 + 6 = 8) or when 1 chemical antagonizes the effect of the second chemical (eg, 0 + 4 = 1). Receptor antagonists are often termed *blockers*. This concept is used to advantage in the clinical treatment of poisoning. For example, the receptor antagonist naloxone is used to treat the respiratory depressive effects of morphine and other morphine-like narcotics by competitive binding to the same receptor. Another example of receptor antagonism is the use of the antiestrogen drug tamoxifen to lower breast cancer risk among women at high risk for this estrogen-related cancer. Tamoxifen competitively blocks estradiol from binding to its receptor. Treatment of organophosphate insecticide poisoning with atropine is an example not of the antidote competing with the poison for the receptor (cholinesterase) but of blocking the receptor (cholinergic receptor) for the excess acetylcholine that accumulates by poisoning of the cholinesterase by the organophosphate (see Chap. 22).

Tolerance

Tolerance is a state of decreased responsiveness to a toxic effect of a chemical resulting from prior exposure to that chemical or to a structurally related chemical. Two major mechanisms are responsible for tolerance: 1 is due to a decreased amount of toxicant reaching the site where the toxic effect is produced (*dispositional tolerance*) and the other is due to a reduced responsiveness of a tissue to the chemical. Comparatively less is known about the cellular mechanisms responsible for altering the responsiveness of a tissue to a toxic chemical than is known about dispositional tolerance. Two chemicals known to produce dispositional tolerance are carbon tetrachloride and cadmium. The barbiturate, phenobarbital, produces tolerance to itself by increasing the expression of enzymes in the liver that are responsible for its biotransformation to pharmacologically inactive products, a process known as "biotransformation enzyme induction." The mechanism of cadmium tolerance is explained by induction of metallothionein, a metal-binding protein. Subsequent binding of cadmium to metallothionein rather than to critical cellular macromolecules decreases its toxicity.

CHARACTERISTICS OF EXPOSURE

Toxic effects in a biological system are not produced by a chemical agent unless that agent or its metabolic breakdown (biotransformation) products reach appropriate sites in the body at a concentration and for a length of time sufficient to produce a toxic manifestation. Many chemicals are of relatively low toxicity in the "native" form but, when acted on by enzymes in the body, are converted to intermediate forms that interfere with normal cellular biochemistry and physiology. Thus, whether a toxic response occurs is dependent on the chemical and physical properties of the agent, the exposure situation, how the agent is metabolized by the system, the concentration of the active form at the particular target site(s), and the overall susceptibility of the biological system or subject. Thus, to characterize fully the potential hazard of a specific chemical agent, we need to know not only what type of effect it produces and the dose required to produce that effect but also information about the agent, the exposure, and its disposition by the subject. Two major factors that influence toxicity as it relates to the exposure situation for a specific chemical are the route of exposure and the duration and frequency of exposure.

Route and Site of Exposure

The major routes (pathways) by which toxic agents gain access to the body are through the gastrointestinal tract (ingestion), the lungs (inhalation), or the skin (topical, percutaneous, or dermal). Toxic

agents generally produce the greatest effect and the most rapid response when given directly into the bloodstream (the intravenous route). An approximate descending order of effectiveness for the other routes would be inhalation, intraperitoneal, subcutaneous, intramuscular, intradermal, oral, and dermal. The "vehicle" (the material in which the chemical is dissolved) and other formulation factors can markedly alter absorption after ingestion, inhalation, or topical exposure. In addition, the route of administration can influence the toxicity of agents. For example, an agent that acts on the CNS, but is efficiently detoxified in the liver, would be expected to be less toxic when given orally than when given via inhalation, because the oral route requires that nearly all of the dose pass through the liver before reaching the systemic circulation and then the CNS.

Occupational exposure to toxic agents most frequently results from breathing contaminated air (inhalation) and/or direct and prolonged contact of the skin with the substance (dermal exposure), whereas accidental and suicidal poisoning occurs most frequently by oral ingestion. Comparison of the toxic dose (TD) of a toxic substance by different routes of exposure often provides useful information about its extent of absorption. In instances when the TD after oral or dermal administration is similar to the TD after intravenous administration, the assumption is that the toxic agent is absorbed readily and rapidly. Conversely, in cases where the TD by the dermal route is several orders of magnitude higher than the oral TD, it is likely that the skin provides an effective barrier to absorption of the agent. Toxic effects by any route of exposure can also be influenced by the concentration of the agent in its vehicle, the total volume of the vehicle and the properties of the vehicle to which the biological system is exposed, and the rate at which exposure occurs. Studies in which the concentration of a chemical in the blood is determined at various times after exposure are often needed to clarify the role of these and other factors in the toxicity of a compound. For more details on the absorption of toxicants, see Chap. 5.

Duration and Frequency of Exposure

Toxicologists usually divide the exposure of experimental animals to chemicals into 4 categories: acute, subacute, subchronic, and chronic. Acute exposure is defined as exposure to a chemical for less than 24 hours, and examples of exposure routes are intraperitoneal, intravenous, and subcutaneous injection; oral intubation; and dermal application. Whereas acute exposure usually refers to a single administration, repeated exposures may be given within a 24-hour period for some slightly toxic or practically nontoxic chemicals. Acute exposure by inhalation refers to continuous exposure for less than 24 hours, most frequently for 4 hours. Repeated exposure is divided into 3 categories: subacute, subchronic, and chronic. *Subacute exposure* refers to repeated exposure to a chemical for 1 month or less, *subchronic* for 1 to 3 months, and *chronic* for more than 3 months, although usually this refers to studies with at least 1 year of repeated dosing. These 3 categories of repeated exposure can be by any route, but most often they occur by the oral route, with the chemical added directly to the diet.

In human exposure situations, the frequency and duration of exposure are usually not as clearly defined as in controlled animal studies, but many of the same terms are used to describe general exposure situations. Thus, workplace or environmental exposures may be described as *acute* (occurring from a single incident or episode), *subchronic* (occurring repeatedly over several weeks or months), or *chronic* (occurring repeatedly for many months or years).

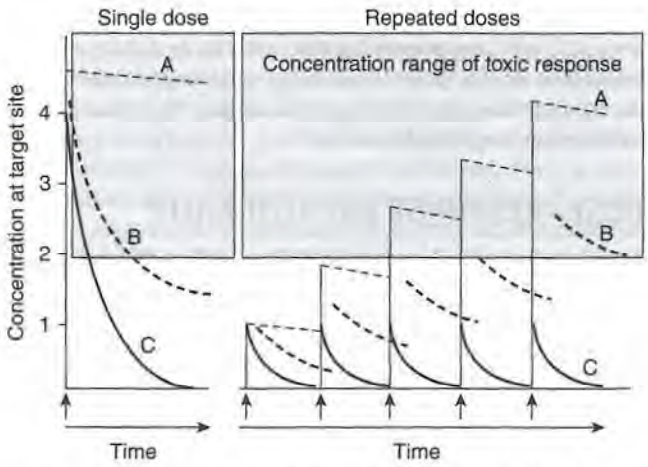

Figure 2-2. *Diagrammatic view of the relationship between dose and concentration at the target site under different conditions of dose frequency and elimination rate.* (Line A) A chemical with very slow elimination (eg, half-life of 1 year). (Line B) A chemical with a rate of elimination equal to frequency of dosing (eg, 1 day). (Line C) Rate of elimination faster than the dosing frequency (eg, 5 hours). Blue shaded area is representative of the concentration of chemical at the target site necessary to elicit a toxic response.

For many chemicals, the toxic effects that follow a single exposure are quite different from those produced by repeated exposure. For example, the primary acute toxic manifestation of benzene is CNS depression, but repeated exposures can result in bone marrow toxicity and an increased risk for leukemia. Acute exposure to chemicals that are rapidly absorbed is likely to produce immediate toxic effects but also can produce delayed toxicity that may or may not be similar to the toxic effects of chronic exposure. Conversely, chronic exposure to a toxic chemical may produce some immediate (acute) effects after each administration in addition to the long-term, low-level, or chronic effects of the toxic substance. In characterizing the toxicity of a specific chemical, it is evident that information is needed not only for the single-dose (acute) and long-term (chronic) effects but also for exposures of intermediate duration. The other time-related factor that is important in the temporal characterization of repeated exposures is the frequency of exposure. The relationship between elimination rate and frequency of exposure is shown in Fig. 2-2. A chemical that produces severe effects with a single dose may have no effect if the same total dose is given in several intervals. For the chemical depicted by line B in Fig. 2-2, in which the half-life for elimination (time necessary for 50% of the chemical to be removed from the bloodstream) is approximately equal to the dosing frequency, a theoretical toxic concentration (shown conceptually as 2 concentration units in Fig. 2-2) is not reached until the fourth dose, whereas that concentration is reached with only 2 doses for chemical A, which has an elimination rate much slower than the dosing interval (time between each repeated dose). Conversely, for chemical C, where the elimination rate is much shorter than the dosing interval, a toxic concentration at the site of toxic effect will never be reached regardless of how many doses are administered. Of course, it is possible that residual cell or tissue damage occurs with each dose even though the chemical itself is not accumulating. The important consideration, then, is whether the interval between doses is sufficient to allow for complete repair of tissue damage. It is evident that with any type of repeated exposure, the production of a toxic effect not only is influenced by the frequency of exposure but may also, in fact, be totally dependent on the frequency rather than the duration of exposure. Chronic toxic effects may occur, therefore, if the chemical accumulates in the biological system (rate

of absorption exceeds the rate of biotransformation and/or excretion), if it produces irreversible toxic effects, or if there is insufficient time for the system to recover from the toxic damage within the exposure frequency interval. For additional discussion of these relationships, see Chaps. 5 and 7.

DOSE–RESPONSE RELATIONSHIP

The characteristics of exposure and the spectrum of toxic effects come together in a correlative relationship customarily referred to as the *dose–response relationship*. Whatever response is selected for measurement, the relationship between the degree of response of the biological system and the amount of toxicant administered assumes a form that occurs so consistently as to be considered the most fundamental and pervasive concept in toxicology.

From a practical perspective, there are 2 types of dose–response relationships: (1) the individual dose–response relationship, which describes the response of an *individual* organism to varying doses of a chemical, often referred to as a "graded" response because the measured effect is continuous over a range of doses, and (2) a quantal dose–response relationship, which characterizes the distribution of individual responses to different doses in a *population* of individual organisms. It is also important to recognize that a given chemical may have multiple different molecular, biochemical, and cellular effects, each with its own "dose–response" relationship. Thus, the nature of a toxic response might very well be different at low doses than at higher doses. In the case of population-level "dose–response" characterization, the observed response is an integration of multiple individual "dose–response relationships" occurring in different cell types, and at different molecular sites within those cells. Subtle effects that occur at low doses may be masked or overwhelmed by more evident responses occurring at higher doses.

Individual, or Graded, Dose–Response Relationships

Individual dose–response relationships are characterized by a dose-related increase in the severity of the response. The dose relatedness of the response often results from an alteration of a specific biochemical process. For example, Fig. 2-3 shows the dose–response relationship between different dietary doses of the organophosphate insecticide chlorpyrifos and the extent of inhibition of 2 different enzymes in the brain and liver: acetylcholinesterase and carboxylesterase. In the brain, the degree of inhibition of both enzymes is clearly dose-related and spans a wide range, although the amount of inhibition per unit dose is different for the 2 enzymes. From the shapes of these 2 dose–response curves it is evident that, in the brain, cholinesterase is more easily inhibited than carboxylesterase. The toxicological response that results is directly related to the degree of cholinesterase enzyme inhibition in the brain. Thus, clinical signs and symptoms for chlorpyrifos would follow a dose–response relationship similar to that for brain cholinesterase. However, as noted above, for many chemicals, more than 1 effect may result because of multiple different target sites in different tissues. Thus, the observed response to varying doses of a chemical in the whole organism is often complicated by the fact that most toxic substances have multiple sites or mechanisms of toxicity, each with its own "dose–response" relationship and subsequent adverse effect. Note that when these dose–response data are plotted using the base 10 log of the dose on the abscissa (Fig. 2.3B), a better "fit" of the data to a straight line usually occurs. This is typical of many graded as well as quantal dose–response relationships.

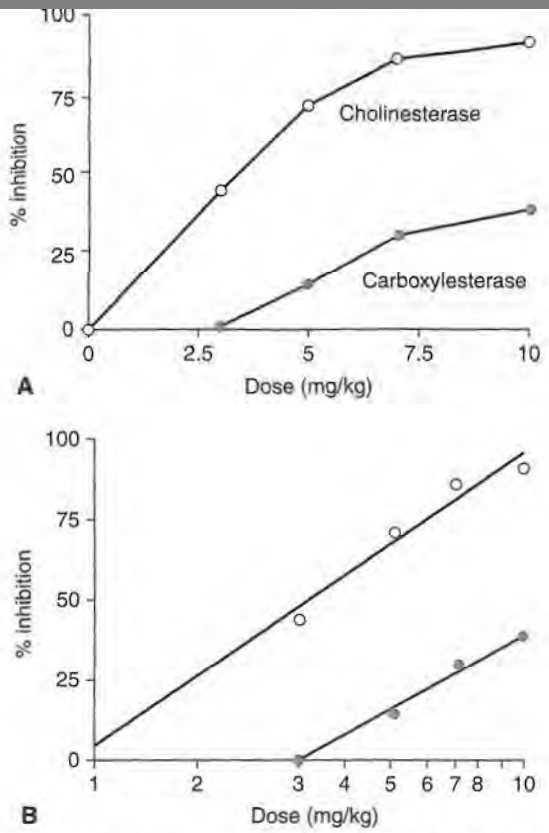

Figure 2-3. *Dose–response relationship between different doses of the organophosphate insecticide chlorpyrifos and esterase enzyme inhibition in the brain.* Open circles and blue lines represent acetylcholinesterase activity and closed circles represent carboxylesterase activity in the brains of pregnant female Long–Evans rats given 5 daily doses of chlorpyrifos. (**A**) Dose–response curve plotted on an arithmetic scale. (**B**) Same data plotted on a semi-log scale. (Data from Lassiter *et al.*, 1999, with permission.)

Quantal Dose–Response Relationships

In contrast to the "graded" or continuous-scale dose–response relationship that occurs in individuals, the dose–response relationships in a *population* are by definition quantal—or "all or none"—in nature, that is, at any given dose, an individual in the population is classified as either a "responder" or a "nonresponder." Although these distinctions of "quantal population" and "graded individual" dose–response relationships are useful, the 2 types of responses are conceptually identical. The ordinate in both cases is simply labeled *the response*, which may be the degree of response in an individual or system or the fraction of a population responding, and the abscissa is the range in administered doses.

A widely used statistical approach for estimating the response of a population to a toxic exposure is the "effective dose" (ED). Generally, the midpoint, or 50%, response level is used, giving rise to the "ED_{50}" value. However, any response level, such as an ED_{01}, ED_{10}, or ED_{30}, could be chosen. A graphical representation of an approximate ED_{50} is shown in Fig. 2-4. Note that these data are "quantal." Where death is the measured end point, the ED_{50} would be referred to as the LD_{50}. Historically, determination of the LD_{50} was often the first experiment performed with a new chemical. Today, it is widely recognized that the LD_{50} is of marginal value as a measure of hazard, although it does provide a useful "ball park" indication of the relative hazard of a compound to cause serious, life-threatening poisoning from a single exposure. Although death is an obvious quantal end point to measure, it should be noted that any

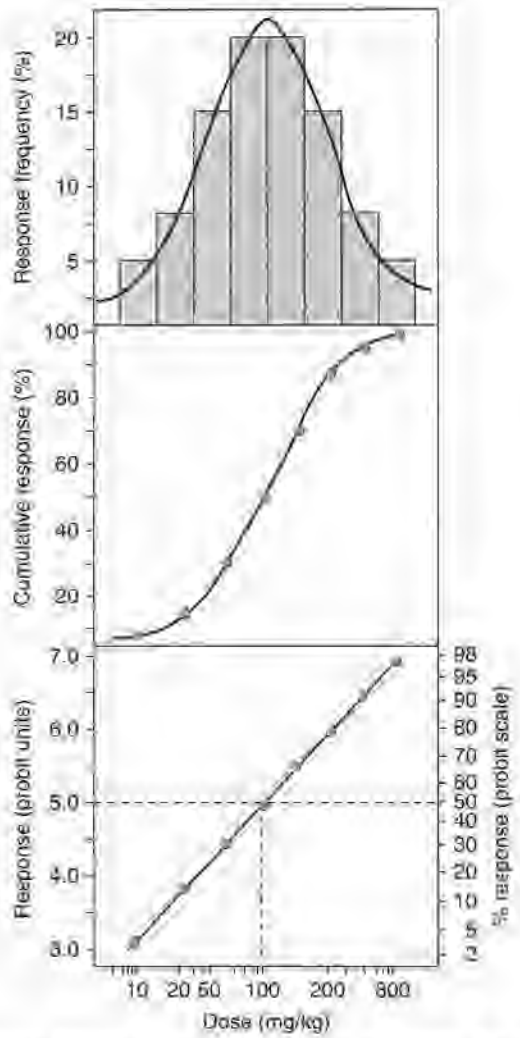

Figure 2-4. *Diagram of quantal dose–response relationship.* The abscissa is a log dosage of the chemical. In the top panel the ordinate is response frequency, in the middle panel the ordinate is percent response, and in the bottom panel the response is in probit units (see text).

quantal response could be used. For example, the LD_{50} of lead or DDT is not a relevant end point when characterizing hazards of the agents to children or wildlife, respectively. Even continuous variables can be converted to quantal responses if desired. For example, an antihypertensive drug that lowers blood pressure might be evaluated in a population by assigning a "responder" as an individual whose blood pressure was lowered by 10 mm Hg or more. Note that, in this example, an individual who responded to a change in blood pressure of 50 mm Hg would classified the same as an individual with a change in only 10 mm Hg, yet an individual with a change in 8 mm Hg would be classified as a "nonresponder." The top panel of Fig. 2-4 shows that quantal dose responses typically exhibit a normal or Gaussian distribution. The frequency histogram in this panel also shows the relationship between dose and effect. The bars represent the percentage of animals that responded at each dose minus the percentage that responded at the immediately lower dose. One can clearly see that only a few animals responded to the lowest dose and the highest dose. Larger numbers of animals responded to doses intermediate between these 2 extremes, and the maximum frequency of response occurred in the middle portion of the dose range. Thus, we have a bell-shaped curve known as a *normal frequency distribution*. The reason for this normal distribution is that there are differences in susceptibility to chemicals among individuals; this is known as biological variation. Animals responding at the left end of the curve are referred to as *hypersusceptible*, and those at the right end of the curve are called *resistant*. If the numbers of individuals responding at each consecutive dose are added together a cumulative, quantal dose–response relationship is obtained. When a sufficiently large number of doses is used with a large number of animals per dose, a sigmoid dose–response curve is observed, as depicted in the middle panel of Fig. 2-4. With the lowest dose (6 mg/kg), 1% of the animals respond. A normally distributed sigmoid curve such as this one approaches a response of 0% as the dose is decreased and approaches 100% as the dose is increased, but—theoretically—it never passes through 0% and 100%. However, the minimally ED of any chemical that evokes a stated all-or-none response is called the *threshold dose* even though it cannot be determined experimentally.

For a normally distributed population response, the sigmoid curve has a relatively linear portion between 16% and 84%. These values represent the limits of 1 standard deviation (SD) of the mean (and the median) in a population with truly normal or Gaussian distribution. However, it is usually not practical to describe the dose–response curve from this type of plot because one does not usually have large enough sample sizes to define the sigmoid curve adequately. In a normally distributed population, the mean ±1 SD represents 68.3% of the population, the mean ±2 SD represents 95.5% of the population, and the mean ±3 SD equals 99.7% of the population. Because quantal dose–response phenomena are usually normally distributed, one can convert the percent response to units of deviation from the mean or normal equivalent deviations (NEDs). Thus, the NED for a 50% response is 0, an NED of +1 is equated with an 84.1% response. Traditionally, units of NED are converted by the addition of 5 to the value to avoid negative numbers; these converted units are called *probit units* (Bliss, 1957). The probit (from the contraction of *prob*ability unit), then, is an NED plus 5. In this transformation, a 50% response becomes a probit of 5, a +1 deviation becomes a probit of 6, and a −1 deviation is a probit of 4.

The data given in the top 2 panels of Fig. 2-4 are replotted in the bottom panel with the response plotted in probit units. The data in the middle panel (which was in the form of a sigmoid curve) and the top panel (a bell-shaped curve) form a straight line when transformed into probit units. In essence, what is accomplished in a probit transformation is an adjustment of quantal data to an assumed normal population distribution, resulting in a straight line. The ED_{50} is obtained by drawing a horizontal line from the probit unit 5, which is the 50% response point, to the dose–effect line. At the point of intersection, a vertical line is drawn, and this line intersects the abscissa at the ED_{50} point. It is evident from the line that information with respect to the ED for 90% or for 10% of the population also may be derived by a similar procedure. Mathematically, it can be demonstrated that the range of values encompassed by the confidence limits is narrowest at the midpoint of the line (ED_{50}) and widest at both extremes (ED_{10} and ED_{90}) of the dose–response curve (dotted lines in Fig. 2-5). In addition to the ED_{50}, the slope of the dose–response curve can also be obtained. Fig. 2-5 demonstrates the dose–response curves for the response of 2 compounds. Compound A exhibits a "flat" dose–response curve, showing that a large change in dosage is required before a significant change in response will be observed. However, compound B exhibits a "steep" dose–response curve, where a relatively small change in dosage will cause a large change in response. It is evident that the ED_{50} for both compounds is the same (8 mg/kg). However, the slopes of the dose–response curves are quite different. At one half of ED_{50} of the compounds (4 mg/kg), less than 1% of the animals exposed to compound B would respond but 20% of the animals given compound A would respond.

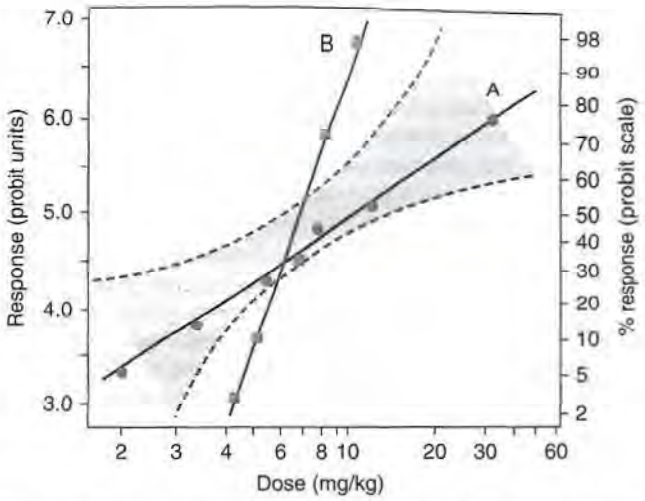

Figure 2-5. *Comparison of dose–response relationship for 2 different chemicals, plotted on a log dose-probit scale.* Note that the slope of the dose–response is steeper for chemical B than for chemical A. Dotted lines represent the confidence limits for chemical A.

In Figs. 2-4 and 2-5 the dosage has been given on a log basis. Although the use of the log of the dosage is empiric, log dosage plots for normally distributed quantal data provide a more nearly linear representation of the data. It must be remembered, however, that this is not universally the case. Some radiation effects, for example, give a better probit fit when the dose is expressed arithmetically rather than logarithmically. There are other situations in which other functions (eg, exponentials) of dosage provide a better fit to the data than does the log function. It is also conventional to express the dosage in milligrams per kilogram. It might be argued that expression of dosage on a mole-per-kilogram basis would be better, particularly for making comparisons among a series of compounds. Although such an argument has considerable merit, dosage is usually expressed in milligrams per kilogram.

One might also view dosage on the basis of body weight as being less appropriate than other bases, such as surface area. The term *allometry* refers to the field of study that examines the relationships between body weight and other biological and physical parameters such as rate of basal metabolism (caloric consumption), heart rate, blood flow, etc. Allometric studies revealed that the relationship between body weight and various other physiological parameters can be closely estimated by the following formula: $Y = aW^b$, where Y is the biological parameter of interest and a and b are constants that relate Y to body weight (Rodricks *et al.*, 2008). In general, organ sizes between species seem to scale best when b is equal to 1, whereas metabolically derived parameters scale better when b is 0.67 to 0.75. The relationship between body surface area and body weight across most mammalian species is closely described by the formula SA = 10.5 × (body weight [grams])$^{0.67}$ (Harkness and Wagner, 1995). Empirical comparisons of toxicity data across species confirm that this relationship is appropriate for toxicological scaling. For example, Travis and White (1988) analyzed a number of toxicity testing data sets for 27 different chemotherapeutic drugs for which toxicity data were available in mouse, rat, hamster, dog, monkey, and human. They found that the exponent of body weight that gave the best correlation with toxicity was 0.73, with 95% confidence bounds of 0.69 to 0.77 (Rodricks *et al.*, 2008). Table 2-2 illustrates the differences in comparative doses when scaling is done by body weight (mg/kg) versus an allometric approach that uses an exponent of either 0.67 or 0.75. Thus, if a scaling factor of $(BW)^{2/3}$ is used, a mouse would need to receive a dose 13 times greater than that required for humans for an equivalent toxic response, whereas the dose would be 7 times greater if a scaling factor of $(BW)^{3/4}$ was used. However, not all toxic responses will necessarily scale across species in the same way. For example, acute lethality seemed to correlate better across species when body weight, rather than body surface area, was used (Rhomberg and Wolff, 1998). The selection of the most appropriate scaling factor should also take into account pharmacokinetic differences, including physiologically based pharmacokinetic modeling (PBPK). When toxicity is attributable to the formation of a toxic metabolite, or when xenobiotic biotransformation is saturated at high doses, a scaling factor of 1 may be more appropriate than 0.75 (Kirman *et al.*, 2003).

Shape of the Dose–Response Curve

Essential Nutrients The shape of the dose–response relationship has many important implications in toxicity assessment. For example, for substances that are required for normal physiological

Table 2-2

Allometric Scaling of Dose Across Different Species

			FOLD DIFFERENCE, RELATIVE TO HUMANS, NORMALIZED BY BODY WEIGHT		
SPECIES	WEIGHT (kg)	SURFACE AREA (cm^2)*	mg/kg	$(BW)^{2/3}$	$(BW)^{3/4}$
Mouse	0.30	103	1	13.0	7.0
Rat	0.2	365	1	6.9	4.3
Guinea pig	0.4	582	1	5.5	3.6
Rabbit	1.5	1410	1	3.5	2.6
Cat	2	1710	1	3.2	2.4
Monkey	4	2720	1	2.6	2.0
Dog	12	5680	1	1.8	1.5
Human	70	18,500	1	1.0	1.0

**Surface area of animals is closely approximated by the following formula: SA = 10.5 × (body weight [grams])$^{2/3}$.*

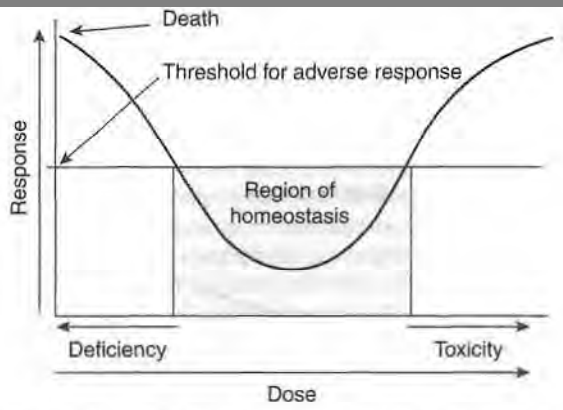

Figure 2-6. *Individual dose–response relationship for an essential substance such as a vitamin or trace element.* It is generally recognized that, for most types of toxic responses, a threshold exists such that at doses below the threshold, no toxicity is evident. For essential substances, doses below the minimum daily requirement, as well as those above the threshold for safety, may be associated with toxic effects. The blue shaded region represents the "region of homeostasis"—the dose range that results in neither deficiency nor toxicity.

function and survival (eg, vitamins and essential trace elements such as chromium, cobalt, and selenium), the "graded" dose–response relationship in an individual over the entire dose range is actually U-shaped (Fig. 2-6). That is, at very low doses, there is a high level of adverse effect, which decreases with an increasing dose. This region of the dose–response relationship for essential nutrients is commonly referred to as a *deficiency*. As the dose is increased to a point where the deficiency no longer exists, no adverse response is detected and the organism is in a state of homeostasis. However, as the dose is increased to abnormally high levels, an adverse response (usually qualitatively different from that observed at deficient doses) appears and increases in magnitude with increasing dose, just as with other toxic substances. Thus, it is recognized that high doses of vitamin A can cause liver toxicity and birth defects, high doses of selenium can affect the brain, and high doses of estrogens may increase the risk of breast cancer, even though low doses of all these substances are essential for life.

Hormesis There is considerable evidence to suggest that some nonnutritional toxic substances may also impart beneficial or stimulatory effects at low doses but that, at higher doses, they produce adverse effects. This concept of "hormesis" was first described for radiation effects but may also pertain to most chemical responses (Calabrese and Blaine, 2005). Thus, in plotting dose versus response over a wide range of doses, the effects of hormesis may also result in a "U-shaped" dose–response curve. In its original development, the concept of hormesis pertained to the ability of substances to stimulate biological systems at low doses but to inhibit them at high doses. The application of the concept of hormesis to whole-animal toxicological dose–response relationships may also be relevant but requires that the "response" on the ordinate be variant with dose. For example, chronic alcohol consumption is well recognized to increase the risk of esophageal cancer, liver cancer, and cirrhosis of the liver at relatively high doses, and this response is dose-related (curve A, Fig. 2-7). However, there is also substantial clinical and epidemiological evidence that low to moderate consumption of alcohol reduces the incidence of coronary heart disease and stroke (curve B, Fig. 2-7) (Hanna *et al.*, 1997). Thus, when all responses are plotted on the ordinate, a "U-shaped" dose–response curve is obtained (curve C, Fig. 2-7). U-shaped dose–response relationships

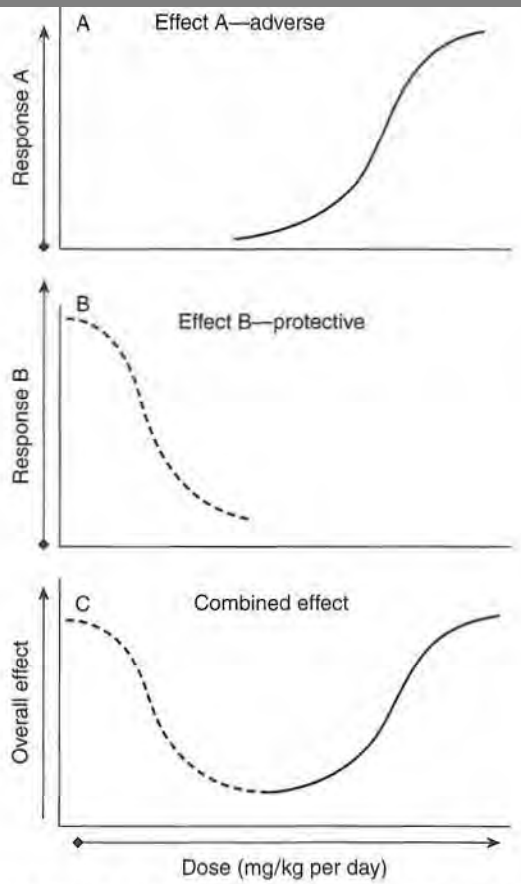

Figure 2-7. *Hypothetical dose–response relationship depicting characteristics of hormesis.* Hormetic effects of a substance are hypothesized to occur when relatively low doses result in the stimulation of a beneficial or protective response (**B**), such as induction of enzymatic pathways that protect against oxidative stress. Although low doses provide a potential beneficial effect, a threshold is exceeded as the dose increases and the net effects will be detrimental (**A**), resulting in a typical dose-related increase in toxicity. The complete dose–response curve (**C**) is conceptually similar to the individual dose–response relationship for essential nutrients shown in Fig. 2-6.

have obvious implications for the process of low-dose extrapolation in risk assessment.

Threshold Another important aspect of the dose–response relationship at low doses is the concept of the threshold. It has long been recognized that acute toxicological responses are associated with thresholds, that is, there is some dose below which the probability of an individual responding is zero. Obviously, the identification of a threshold depends on the particular response that is measured, the sensitivity of the measurement, and the number of subjects studied. For the individual dose–response relationship, thresholds for most toxic effects certainly exist, although interindividual variability in response and qualitative changes in response pattern with dose make it difficult to establish a true "no effects" threshold for any chemical. The biological basis of thresholds for acute responses is well established and frequently can be demonstrated on the basis of mechanistic information (Aldridge, 1986). The traditional approaches to establishing acceptable levels of exposure to chemicals are inherently different for threshold versus nonthreshold responses. The existence of thresholds for chronic responses is less well defined, especially in the area of chemical carcinogenesis. It is, of course, impossible to scientifically prove the absence of a threshold, as one can never prove a negative.

Nevertheless, for the identification of "safe" levels of exposure to a substance, the absence or presence of a threshold is important for practical reasons (see Chap. 4). A classic example of the difficulty of establishing thresholds experimentally is provided by the "ED_{01}" study, where over 24,000 mice and 81 different treatment groups were used to determine the shape of the dose–response relationship for the prototypical carcinogen 2-acetylaminofluorene (2-AAF). The study was designed to identify a statistically significant response of 1% (0.01 probability). The mice were exposed to 2-AAF at 1 of 7 different doses in the dose range of 30 to 150 ppm (plus 0 dose control) (Littlefield *et al.*, 1979). Eight "sacrifice intervals" were used to determine how quickly tumors developed. Both types of tumors demonstrated increasing incidence with increasing dose, but the shapes of the 2 curves are dramatically different. For liver tumors, no clear threshold was evident, whereas for bladder tumors, an apparent threshold was evident. However, the apparent threshold, or "no observable adverse effect level" (NOAEL), for bladder cancer was lower at 33 months (45 ppm) than at 24 months (75 ppm). Of course, the ability to detect a low incidence of tumors depends on the number of animals used in the study. Thus, although a threshold (a dose below which no response occurs) appears evident for bladder tumors, one cannot say for certain that tumors would not occur if more animals had been included in the lower-dose groups. A different animal model that relies on relatively brief exposure of rainbow trout embryos to carcinogens has allowed an even more statistically stringent analysis of the shape of the dose–response curve at low doses for mutagenic carcinogens. Using this model with 2 different genotoxic carcinogens, dibenzo[*d,e,f,p*]chrysene (DBC, also referred to as dibenzo[*a,l*]pyrene) and aflatoxin B_1 (AFB_1), estimates of the shape of the dose–response curve down to a response level of 1 additional tumor in 5000 animals could be obtained, because very large numbers of animals could be exposed. In both studies, over 40,000 trout were exposed to different doses ranging over a factor of 200 (AFB_1, lowest dose 0.5 ppb, highest dose 110 ppb) to 500 (DBC, lowest dose 0.45 ppm, highest dose 225 ppm), with over 8000 animals in the control and low-dose groups (Williams, 2012). Both of these chemicals are potent mutagens, so it was assumed that both the rate of DNA adduct formation and the tumor incidence would be linear throughout the dose range. However, for DBC, there was a clear deviation from linearity at the lower doses, such that the extrapolated dose–response curve crossed the *y*-axis at 1 cancer in a million exposed animals at a dose 500- to 1500-fold (depending on the statistical model) higher than would have been predicted from the linear extrapolation below the 10% response range (ED_{10}) (Bailey *et al.*, 2009) (Fig. 2-8). Remarkably, although the tumor response exhibited a clear "threshold," the formation of DBC–DNA adducts was quite linear through the lowest dose used. In contrast, in a similarly designed study using the potent carcinogen, AFB_1, both tumor response and AFB–DNA adduct formation appear approximately linear down through the lowest dose; the liver tumor response to AFB_1 remained linear to the lowest dose, although the slope was about 1.5 and the predicted dose resulting in 1 cancer in a million exposed animals was about 10-fold higher than that predicted from the extrapolated LED_{10} line, although the lowest doses tested yielded tumor incidence that was close (within a factor of 2) to the background tumor rate (Fig. 2-9) (Williams *et al.*, 2009a; Williams, 2012).

(See Chap. 4 for more discussion on statistical issues related to extrapolation of dose–response curves and the determination of NOAELs.)

In evaluating the shape of the dose–response relationship in populations, it is realistic to consider inflections in the shape of the dose–response curve rather than absolute thresholds. That is, the slope of the dose–response relationship at high doses may be substantially different from the slope at low doses, usually because of dispositional differences in the chemical. Saturation of biotransformation pathways, protein-binding sites or receptors, and depletion of intracellular cofactors represent some reasons why sharp inflections in the dose–response relationship may occur. For example, the widely used analgesic acetaminophen has a very low rate of liver toxicity at normal therapeutic doses. Even though a toxic metabolite (*N*-acetyl-*p*-benzoquinone imine [NAPQI]) is produced in the liver

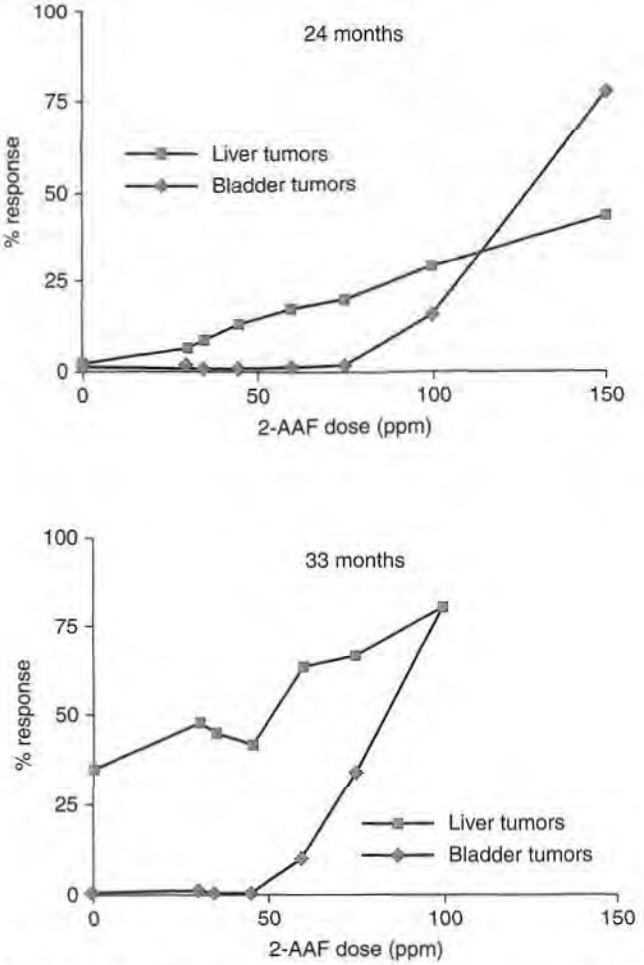

Figure 2-8. *Dose–response relationship for carcinogens—rodents and 2-AAF.*

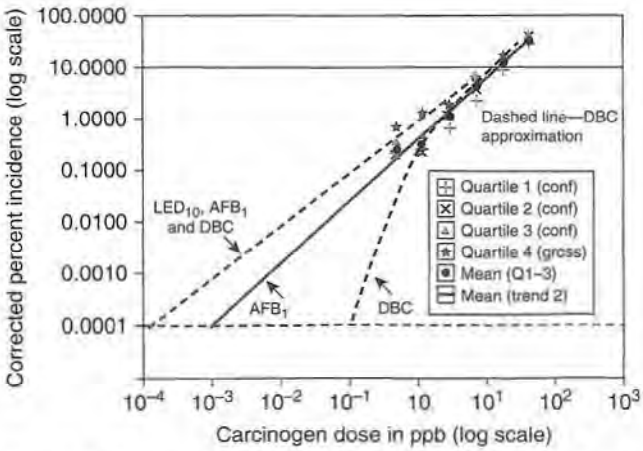

Figure 2-9. *Dose–response relationship for carcinogens—fish and aflatoxin B1.* (Reproduced with permission from Williams, 2012.)

at therapeutic doses, it is rapidly detoxified through conjugation with the intracellular antioxidant glutathione. However, at very high doses, the level of intracellular glutathione in the liver is depleted and NAPQI accumulates, causing serious and potentially fatal liver toxicity. This effect is analogous to the rapid change in pH of a buffered solution that occurs when the buffer capacity is exceeded. Some toxic responses, most notably the development of cancer after the administration of genotoxic carcinogens, are often considered to be linear at low doses and thus do not exhibit a threshold. In such circumstances, there is no dose with "zero" risk, although the risk decreases proportionately with a decrease in the dose. The existence or lack of existence of a threshold dose for carcinogens has many regulatory implications and is a point of considerable controversy and research in the field of quantitative risk assessment for chemical carcinogens (see Chap. 4).

Nonmonotonic Dose–Response Curves For chemicals that exert their primary toxic effects via modification of hormonal responses (endocrine disruptors), it is possible that effects occur at relatively low doses that are not seen at higher doses, thereby seemingly defying the traditional concept of "dose–response".

The characterization of so-called nonmonotonic dose–response (NMDR) curves is an important refinement in our understanding of dose–response relationships in toxicology (Fig. 2-10). Indeed, some chemicals, such as the plastics monomer bisphenol A (BPA), exhibit relatively little evident toxicity at high doses in traditional acute toxicity testing procedures, yet may have important biological effects when exposure occurs during sensitive periods of development, even at doses well below those shown to cause evident toxicity. For example, human pituitary cells cultured in the presence of BPA elicited significant responses at concentrations of 0.001 and

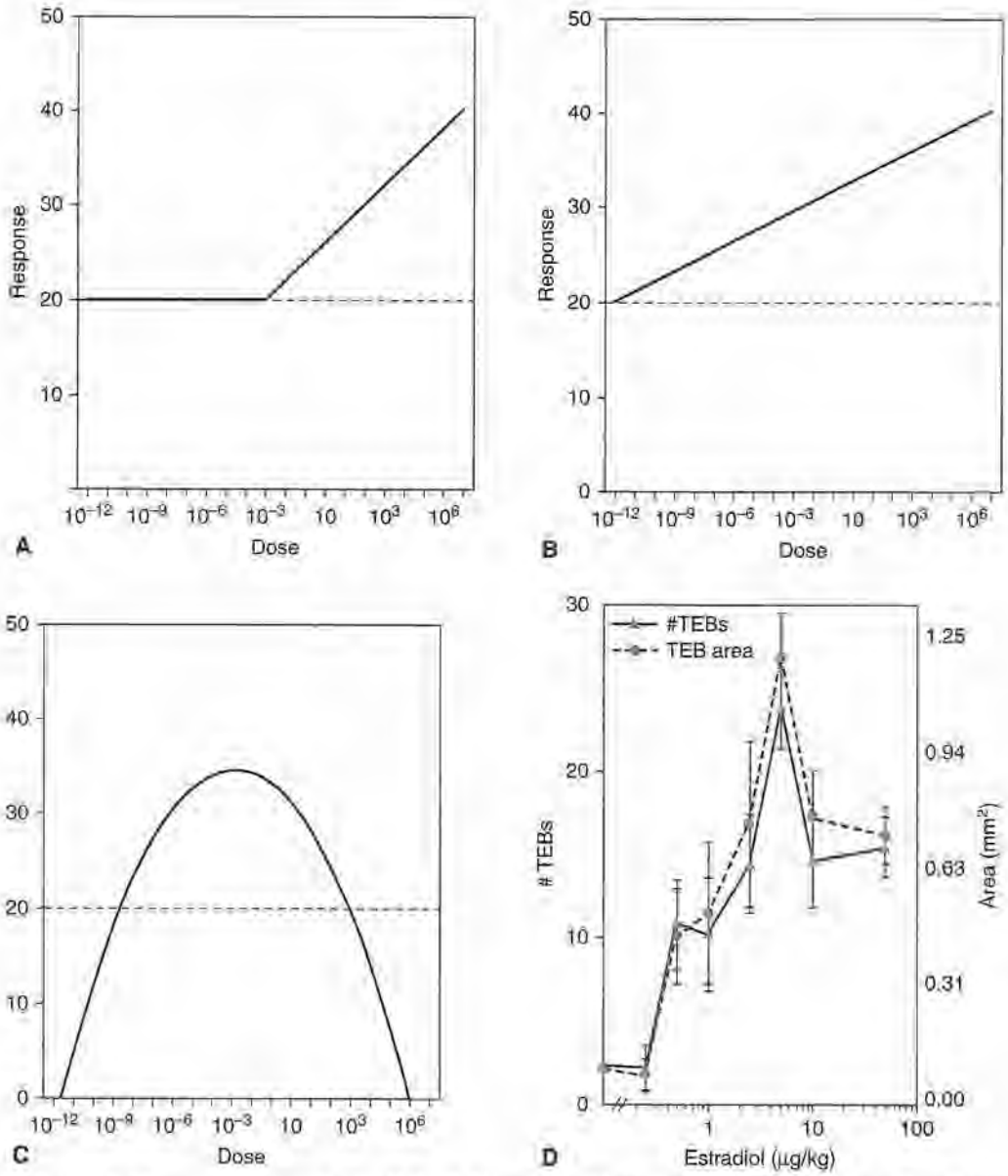

Figure 2-10. *Hypothetical dose–response curves for the (A) threshold responses, (B) nonthreshold linear response, and (C and D) nonmonotonic dose–response (NMDR).* Curves A and B reflect traditional dose–response relationships. However, in the NMDR curve (C), an increase in dose does not necessarily correspond to an increase in response, such that, in this example, doses from 10^{-12} to 10^{-3} dose units result in an increase in response, and doses from 10^{-1} to 10^{6} dose units result in a decrease in response. Curve D represents the NMDR curves observed in mammary gland morphological parameters after administration of estradiol to ovariectomized females. The *left y-axis* is the number of terminal end buds (TEBs), and the *right y-axis* is total area of all TEBs; the TEB is an estrogen-dependent structure. (Based on Vandenberg *et al.*, 2009.)

0.01 nM, but not at 1 and 10 nM, yet the response was seen at 100 nM (Vandenberg *et al.*, 2009). Several other studies have found that BPA and other endocrine-active xenobiotics can elicit NMDR relationships for a variety of other specific receptors and/or cell signaling pathways (reviewed in Vandenberg *et al.*, 2009).

Specific cellular/molecular mechanisms that might explain NMDR curves include: (1) upregulation of some receptors at low concentrations, with downregulation of the same receptors at higher levels, and/or (2) integration of 2 or more monotonic dose–response curves that occur through different molecular/cellular pathways with common end points but opposite effects (Vandenberg *et al.*, 2009). Since endocrine-active xenobiotics may act as weak agonists for specific hormone receptors, it is reasonable that low doses could have different effects than high doses if, as partial agonists, they competitively inhibit endogenous ligands at higher concentrations but have either no or positive agonist effects at low concentrations. Another explanation for NMDR curves is that we simply do not understand all the varied and interconnected molecular pathways that work in concert to produce an observable response at the organismal level. Indeed, BPA has been shown to have multiple different effects on a myriad of putative molecular pathways involved in hormone function, so it perhaps is not surprising to see NMDR functions over dose ranges of many orders of magnitude (Vandenberg *et al.*, 2009).

Assumptions in Deriving the Dose–Response Relationship

A number of assumptions must be considered before dose–response relationships can be used appropriately. The first is that the response is due to the chemical administered. To describe the relationship between a toxic material and an observed effect or response, one must know with reasonable certainty that the relationship is indeed a causal one. For some data, it is not always apparent that the response is a result of chemical exposure. For example, an epidemiological study might result in the discovery of an "association" between a response (eg, disease) and 1 or more variables. Frequently, the data are presented similarly to the presentation of "dose response" in pharmacology and toxicology. Use of the dose response in this context is suspect unless other convincing evidence supports a causal connection between the estimated dose and the measured end point (response). Unfortunately, in nearly all retrospective and case–control studies and even in many prospective studies, the dose, duration, frequency, and routes of exposure are seldom quantified, and other potential etiologic factors are frequently present. In its most strict usage, then, the dose–response relationship is based on the knowledge that the effect is a result of a known toxic agent or agents.

A second assumption seems simple and obvious: the magnitude of the response is in fact related to the dose. Perhaps because of its apparent simplicity, this assumption is often a source of misunderstanding. It is really a composite of 3 other assumptions that recur frequently:

1. There is a molecular target site (or sites) with which the chemical interacts to initiate the response.
2. The production of a response and the degree of response are related to the concentration of the chemical at the target site.
3. The concentration at the site is, in turn, related to the dose administered.

The third assumption in using the dose–response relationship is that there exist both a quantifiable method of measuring and a precise means of expressing the toxicity. For any given dose–response relationship, a great variety of criteria or end points of toxicity could be used. The ideal criterion would be one closely associated with the molecular events resulting from exposure to the toxicant. It follows from this that a given chemical may have a family of dose–response relationships, 1 for each toxic end point. For example, a chemical that produces cancer through genotoxic effects, liver damage through inhibition of a specific enzyme, and CNS effects via a different mechanism, may have 3 distinct dose–response relationships, 1 for each end point. Early in the assessment of toxicity, little mechanistic information is usually available; thus, establishing a dose–response relationship based on the molecular mechanism of action is usually impossible. Indeed, it might not be approachable even for well-known toxicants. In the absence of a mechanistic, molecular ideal criterion of toxicity, one looks to a measure of toxicity that is unequivocal and clearly relevant to the toxic effect. Such measures are often referred to as "effects-related biomarkers." For example, with a new compound chemically related to the class of organophosphate insecticides, one might approach the measurement of toxicity by measuring the inhibition of cholinesterase in blood. In this way, one would be measuring, in a readily accessible system and using a technique that is convenient and reasonably precise, a prominent effect of the chemical and one that is usually pertinent to the mechanism by which toxicity is produced.

The selection of a toxic end point for measurement is not always so straightforward. Even the example cited above may be misleading, as an organophosphate may produce a decrease in blood cholinesterase, but this change may not be directly related to its toxicity. As additional data are gathered to suggest a mechanism of toxicity for any substance, other measures of toxicity may be selected. Although many end points are quantitative and precise, they are often indirect measures of toxicity. Changes in enzyme levels in blood can be indicative of tissue damage. For example, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) are used to detect liver damage. Use of these enzymes in serum is yet another example of an effects-related biomarker because the change in enzyme activity in the blood is directly related to damage to liver cells. Much of clinical diagnostic medicine relies on effects-related biomarkers, but to be useful the relationship between the biomarker and the disease must be carefully established. Patterns of isozymes and their alteration may provide insight into the organ or system that is the site of toxic effects. As discussed later in this chapter, the new tools of toxicogenomics provide an unprecedented opportunity to discover new "effects-related biomarkers" in toxicology.

Many direct measures of effects are also not necessarily related to the mechanism by which a substance produces harm to an organism but have the advantage of permitting a causal relation to be drawn between the chemical and its action. For example, measurement of the alteration of the tone of smooth or skeletal muscle for substances acting on muscles represents a fundamental approach to toxicological assessment. Similarly, measures of heart rate, blood pressure, and electrical activity of heart muscle, nerve, and brain are examples of the use of physiological functions as indices of toxicity. Measurement can also take the form of a still higher level of integration, such as the degree of motor activity or behavioral change.

The measurements used as examples in the preceding discussion all assume prior information about the toxicant, such as its target organ or site of action or a fundamental effect. However, such information is usually available only after toxicological screening and testing based on other measures of toxicity. With a new substance, the customary starting point is a single dose acute toxicity test designed to provide preliminary identification of target organ

toxicity. Studies specifically designed with lethality as an end point are no longer recommended by the United States or international agencies. Data from acute studies provide essential information for choosing doses for repeated dosing studies as well as choosing specific toxicological end points for further study. Key elements of the study design must be a careful, disciplined, detailed observation of the intact animal extending from the time of administration of the toxicant to any clinical signs of distress, which may include detailed behavioral observations or physiological measures. It is recommended that these observations be taken over a 14-day period. From properly conducted observations, immensely informative data can be gathered by a trained toxicologist. Second, an acute toxicity study ordinarily is supported by histological examination of major tissues and organs for abnormalities. From these observations, one can usually obtain more specific information about the events leading to the various end points, the target organs involved, and often a suggestion about the possible mechanism of toxicity at a relatively fundamental level.

Evaluating the Dose–Response Relationship

Comparison of Dose Responses Fig. 2-11 illustrates a hypothetical quantal dose–response curve for a desirable effect of a chemical (effective dose, ED) such as anesthesia, a toxic effect (toxic dose, ED) such as liver injury, and the lethal dose (LD). As depicted in Fig. 2-11, a parallelism is apparent between the ED curve and the curve depicting mortality (LD). It is tempting to view the parallel dose–response curves as indicative of identity of mechanism—that is, to conclude that the lethality is a simple extension of the therapeutic effect. Whereas this conclusion may ultimately prove to be correct in any particular case, it is not warranted solely on the basis of the 2 parallel lines. The same admonition applies to any pair of parallel "effect" curves or any other pair of toxicity or lethality curves.

Therapeutic Index The hypothetical curves in Fig. 2-11 illustrate 2 other interrelated points: the importance of the selection of the toxic criterion and the interpretation of comparative effect. The concept of the "therapeutic index" (TI), which was introduced by Paul Ehrlich in 1913, can be used to illustrate this relationship. Although the TI is directed toward a comparison of the therapeutically ED to the TD of a chemical, it is equally applicable to considerations of comparative toxicity. The TI in its broadest sense is defined as the ratio of the dose required to produce a toxic effect to the dose needed to elicit the desired therapeutic response. Similarly, an index of comparative toxicity is obtained by the ratio of doses of 2 different materials to produce an identical response or the ratio of doses of the same material necessary to yield different toxic effects.

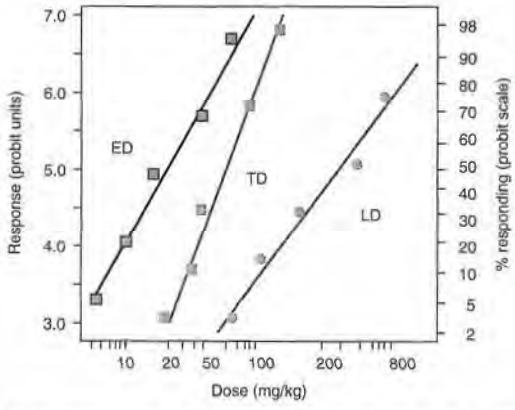

Figure 2-11. *Comparison of effective dose (ED), toxic dose (TD), and lethal dose (LD).* The plot is of log dosage versus percentage of population responding in probit units.

The most commonly used index of effect, whether beneficial or toxic, is the median effect dose (ED_{50}). The TI of a drug is an approximate statement about the relative safety of a drug expressed as the ratio of the adverse end point or TD (historically the LD) to the therapeutic dose:

$$TI = \frac{TD_{50}}{ED_{50}}.$$

From Fig. 2-11 one can approximate a TI by using these median doses. The larger the ratio, the greater is the relative safety. The ED_{50} is approximately 20, and the TD_{50} is about 60; thus, the TI is 3, a number indicating that reasonable care in exposure to the drug is necessary to avoid toxicity. However, the use of the median effective and median toxic doses is not without disadvantages, because median doses tell nothing about the slopes of the dose–response curves for therapeutic and toxic effects.

Margins of Safety and Exposure One way to overcome this deficiency is to use the ED_{99} for the desired effect and the TD_1 for the undesired effect. These parameters are used in the calculation of the margin of safety (MOS):

$$MOS = \frac{TD_1}{ED_{99}}.$$

The quantitative comparisons described above have been used mainly after a single administration of chemicals. However, for chemicals for which there is no beneficial or effective dose and exposures are likely to occur repeatedly, the ratio of TD_1 to ED_{99} has little relevance. Thus, for nondrug chemicals, the term *MOS* has found use in risk assessment procedures as an indicator of the magnitude of the difference between an estimated "exposed dose" to a human population and the NOAEL or other benchmark dose determined in experimental animals.

A measure of the degree of accumulation of a chemical and/or its toxic effects can also be estimated from quantal toxicity data. The *chronicity index* of a chemical is a unitless value obtained by dividing its 1-dose TD_{50} by its 90-dose (90-day) TD_{50}, with both expressed in milligrams per kilogram per day. Theoretically, if no cumulative effect occurs over the doses, the chronicity index will be 1. If a compound were absolutely cumulative, the chronicity index would be 90.

Historically, statistical procedures similar to those used to calculate the LD_{50} can also be used to determine the lethal time 50 (LT_{50}), or the time required for half the animals to die (Litchfield, 1949). The LT_{50} value for a chemical indicates the time course of the toxic effects but does not indicate whether 1 chemical is more toxic than another.

Frequently, dose–response curves from repeated-dose experimental animal studies (subacute, subchronic, or chronic) are used to estimate the NOAEL, or some other "benchmark" measure of minimal toxic response, such as the dose estimated to produce toxic effects in 10% of the population (TD_{10}) (see also Chap. 4). These estimates of minimal TD, derived from quantal dose–response curves, can be used in risk assessment to derive a "margin of exposure" (MOE) index. This index compares the estimated daily exposure, in milligrams per kilogram per day, that might occur under a given set of circumstances with some estimated value from the quantal dose–response relationship (eg, NOAEL or TD_{10}). Like the

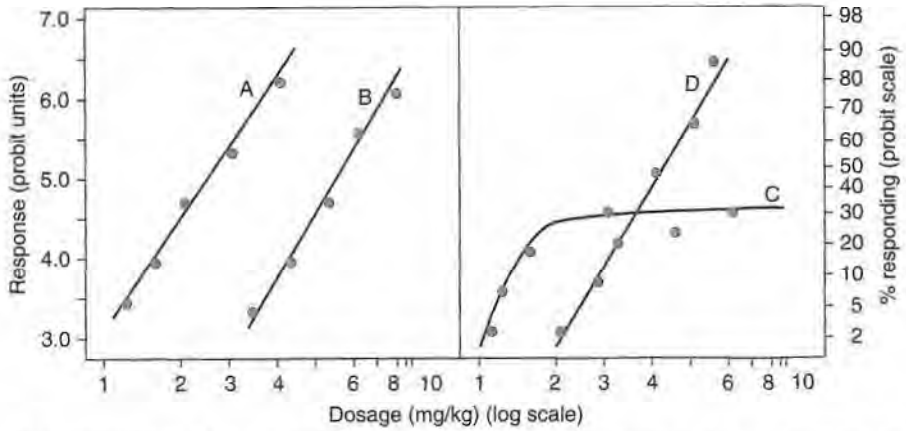

Figure 2-12. *Schematic representation of the difference in the dose–response curves for 4 chemicals (**A-D**), illustrating the difference between potency and efficacy (see text).*

MOS, the MOE is often expressed as a ratio of these 2 values. Thus, for example, if an estimate of human exposure to a pesticide residue yielded a value of 0.001 mg/kg per day, and a TD_{10} of 1 mg/kg per day was determined for that same pesticide, the MOE would be 1000. This value indicates that the estimate of daily exposure under the described set of conditions is 1/1000 the estimated daily dose that would cause evident toxicity in 10% of exposed animals. (See Chap. 4 for a more complete discussion of benchmark doses, NOAELs, and MOE.)

Potency versus Efficacy To compare the toxic effects of 2 or more chemicals, the dose response to the toxic effects of each chemical must be established. One can then compare the potency and maximal efficacy of the 2 chemicals to produce a toxic effect. These 2 important terms can be explained by reference to Fig. 2-12, which depicts dose–response curves to 4 different chemicals for the frequency of a particular toxic effect, such as the production of tumors. Chemical A is said to be more potent than chemical B because of their relative positions along the dosage axis. Potency thus refers to the range of doses over which a chemical produces increasing responses. Thus, A is more potent than B and C is more potent than D. Maximal efficacy reflects the limit of the dose–response relationship on the response axis to a certain chemical. Chemicals A and B have equal maximal efficacy, whereas the maximal efficacy of C is less than that of D.

VARIATION IN TOXIC RESPONSES

Selective Toxicity

Selective toxicity means that a chemical produces injury to 1 kind of living matter without harming another form of life even though the 2 may exist in intimate contact (Albert, 1973). The living matter that is injured is termed the *uneconomic form* (or undesirable), and the matter protected is called the *economic form* (or desirable). They may be related to each other as parasite and host or may be 2 tissues in 1 organism. This biological diversity interferes with the ability of ecotoxicologists to predict the toxic effects of a chemical in 1 species (humans) from experiments performed in another species (laboratory animals). However, by taking advantage of the biological diversity, it is possible to develop chemicals that are lethal for an undesired species and harmless for other species. In agriculture, for example, there are fungi, insects, and even competitive plants that injure the crop, and thus selective pesticides are needed. Similarly, animal husbandry and human medicine require chemicals, such as antibiotics, that are selectively toxic to the undesirable form but do not produce damage to the desirable form.

Drugs and other chemicals used for selective toxic purposes are selective for 1 of 2 reasons. Either (1) the chemical is equally toxic to both economic and uneconomic cells but is accumulated mainly by uneconomic cells or (2) it reacts fairly specifically with a cytological or a biochemical feature that is absent from or does not play an important role in the economic form (Albert, 1973). Selectivity resulting from differences in distribution usually is caused by differences in the absorption, biotransformation, or excretion of the toxicant. The selective toxicity of an insecticide spray may be partly due to a larger surface area per unit weight that causes the insect to absorb a proportionally larger dose than does the mammal being sprayed. The effectiveness of radioactive iodine in the treatment of hyperthyroidism (as well as its thyroid carcinogenicity) is due to the selective ability of the thyroid gland to accumulate iodine. A major reason why chemicals are toxic to one, but not to another, type of tissue is that there are differences in accumulation of the ultimate toxic compound in various tissues. This, in turn, may be due to differences in the ability of various tissues to transport or biotransform the chemical into the ultimate toxic product.

Selective toxicity caused by differences in comparative cytology is exemplified by a comparison of plant and animal cells. Plants differ from animals in many ways—for example, absence of a nervous system, an efficient circulatory system, and muscles as well as the presence of a photosynthetic mechanism and cell walls. The fact that bacteria contain cell walls and humans do not has been utilized in developing selective toxic chemotherapeutic agents, such as penicillin and cephalosporins, that kill bacteria but are relatively *non*toxic to mammalian cells.

Selective toxicity can also be a result of a difference in biochemistry in the 2 types of cells. For example, bacteria do not absorb folic acid but synthesize it from *p*-aminobenzoic acid, glutamic acid, and pteridine, whereas mammals cannot synthesize folic acid but have to absorb it from the diet. Thus, sulfonamide drugs are selectively toxic to bacteria because the sulfonamides, which resemble *p*-aminobenzoic acid in both charge and dimensions, antagonize the incorporation of *p*-aminobenzoic acid into the folic acid molecule—a reaction that humans do not carry out.

Species Differences

Although a basic tenet of toxicology is that "experimental results in animals, when properly qualified, are applicable to humans," it is important to recognize that both quantitative and qualitative

differences in response to toxic substances may occur among different species. As discussed above, there are many reasons for selective toxicity among different species. Even among phylogenetically similar species (eg, rats, mice, guinea pigs, and hamsters), large differences in response may occur. For example, the LD_{50} for the highly toxic dioxin, TCDD, differs by more than 1000-fold between guinea pigs and hamsters. Not only the lethal dose for TCDD but also the particular target organs affected vary widely among species. Species differences in response to carcinogenic chemicals represent an important issue in regulatory risk assessment. As discussed in Chap. 4, extrapolation of laboratory animal data to infer human cancer risk is currently a key component of regulatory decision making. The validity of this approach of course depends on the relevance of the experimental animal model to humans. Large differences in carcinogenic response between experimental animal species are not unusual. For example, mice are highly resistant to the hepatocarcinogenic effects of the fungal toxin AFB_1. Dietary doses as high as 10,000 ppb failed to produce liver cancer in mice, whereas in rats dietary doses as low as 15 ppb produced a significant increase in liver tumors (Wogan *et al.*, 1974). The mechanistic basis for this dramatic difference in response appears to be entirely related to species differences in the expression of a particular form of glutathione *S*-transferase (mGSTA3-3) that has unusually high catalytic activity toward the carcinogenic epoxide of aflatoxin (Eaton and Gallagher, 1994). Mice express this enzyme constitutively, whereas rats normally express a closely related form with much less detoxifying activity toward aflatoxin epoxide. Interestingly, rats do possess the gene for a form of glutathione *S*-transferase with high catalytic activity toward aflatoxin epoxide (rGSTA5-5) that is inducible by certain dietary antioxidants and drugs. Thus, dietary treatment can dramatically change the sensitivity of a species to a carcinogen.

Other examples in which large species differences in response to carcinogens have been observed include the development of renal tumors from 2,3,5-trimethylpentane and D-limonene in male rats (Lehman-McKeeman and Caudill, 1992), the production of liver tumors from "peroxisomal proliferators" such as the antilipidemic drug clofibrate and the common solvent trichloroethylene (Roberts, 1999), and the induction of nasal carcinomas in rats after inhalation exposure to formaldehyde (Monticello and Morgan, 1997).

Identifying the mechanistic basis for species differences in response to chemicals is an important part of toxicology because only through a thorough understanding of these differences can the relevance of animal data to human response be verified.

Individual Differences in Response

Even within a species, large interindividual differences in response to a chemical can occur because of subtle genetic differences. Hereditary differences in a single gene that occur in more than 1% of the population are referred to as *genetic polymorphism* and may be responsible for idiosyncratic reactions to chemicals, as discussed earlier in this chapter. However, genetic polymorphism may have other important but less dramatic effects than those described for acute idiosyncratic responses (such as that occurring in pseudocholinesterase-deficient individuals after succinylcholine exposure). For example, it is recognized that approximately 50% of the Caucasian population has a gene deletion for the enzyme glutathione *S*-transferase M1. This enzyme has no apparent significant physiological function, and thus homozygotes for the gene deletion (eg, those who lack both copies of the normal gene) are functionally and physiologically normal. However, epidemiological studies have indicated that smokers who are homozygous for the null allele may be at slightly increased risk of developing lung cancer compared with smokers who have 1 or both copies of the normal gene (Mohr *et al.*, 2003). Chap. 6 provides additional examples of genetic differences in biotransformation enzymes that may be important determinants of variability in individual susceptibility to chemical exposures.

Genetic polymorphism in physiologically important genes may also be responsible for interindividual differences in toxic responses. For example, studies in transgenic mice have shown that mice possessing 1 copy of a mutated *p53* gene (a so-called tumor suppressor gene; see Chap. 8) are much more susceptible to some chemical carcinogens than are mice with 2 normal copies of the gene (Tennant *et al.*, 1999). In humans, there is evidence that possessing 1 mutated copy of a tumor suppressor gene greatly increases the risk of developing certain cancers. For example, retinoblastoma is a largely inherited form of cancer that arises because of the presence of 2 copies of a defective tumor suppressor gene (the Rb gene) (Wiman, 1993). Individuals with 1 mutated copy of the Rb gene and 1 normal copy are not destined to acquire the disease (as are those with 2 copies of the mutated gene), although their chance of acquiring it is much greater than that of persons with 2 normal Rb genes. This is the case because both copies of the gene must be nonfunctional for the disease to develop. With 1 mutated copy present genetically, the probability of acquiring a mutation of the second gene (potentially from exposure to environmental mutagens) is much greater than the probability of acquiring independent mutations in both copies of the gene as would be necessary in people with 2 normal Rb alleles. (See Chap. 8 for additional discussion of tumor suppressor genes.)

As our understanding of the human genome increases, more "susceptibility" genes will be discovered, and it is likely that the etiology of many chronic diseases will be shown to be related to a combination of genetics and environment. Simple blood tests may ultimately be developed that allow an individual to learn whether he or she may be particularly susceptible to specific drugs or environmental pollutants. Although the public health significance of this type of information could be immense, the disclosure of such information raises many important ethical and legal issues that must be addressed before wide use of such tests.

The study of "gene–environment" interactions, or "ecogenetics" (Costa and Eaton, 2006), is a rapidly developing field of substantial relevance to toxicology. It is likely that the majority of chronic diseases develop as a result of the complex interplay between multiple genes and the myriad of environmental factors, including diet, lifestyle, and occupational and/or environmental exposures to toxic substances. The growing field of epigenetics, discussed in more detail later in this chapter, is likely to have an equally great impact on the science of toxicology, as it is likely that many xenobiotics will be found to exert many of their chronic adverse effects through subtle effects on gene expression.

DESCRIPTIVE ANIMAL TOXICITY TESTS

Two main principles underlie all descriptive animal toxicity testing. The first is that the effects produced by a compound in laboratory animals, when properly qualified, are applicable to humans. This premise applies to all of experimental biology and medicine. Most, if not all, known chemical carcinogens in humans are carcinogenic in some species, but not necessarily in all species of laboratory animals. It has become increasingly evident that the converse—that all chemicals identified as carcinogenic in laboratory animals are also carcinogenic in humans—is not true (Dybing and Sanner, 1999; Grisham, 1997; Hengstler *et al.*, 1999). However, for regulatory

and risk assessment purposes, positive carcinogenicity tests in animals are usually interpreted as indicative of potential human carcinogenicity. If a clear understanding of the mechanism of action of the carcinogen indicates that a positive response in animals is not relevant to humans, a positive animal bioassay may be considered irrelevant for human risk assessment (see Chap. 4). This species variation in carcinogenic response appears to be due in many instances to differences in biotransformation of the procarcinogen to the ultimate carcinogen (see Chap. 6).

The second principle is that exposure of experimental animals to chemicals in high doses is a necessary and valid method of discovering possible hazards in humans. This principle is based on the quantal dose–response concept that the incidence of an effect in a population is greater as the dose or exposure increases. Practical considerations in the design of experimental model systems require that the number of animals used in toxicology experiments always be small compared with the size of human populations at risk. Obtaining statistically valid results from such small groups of animals requires the use of relatively large doses so that the effect will occur frequently enough to be detected. However, the use of high doses can create problems in interpretation if the response(s) obtained at high doses does not occur at low doses. Thus, for example, it has been shown that bladder tumors observed in rats fed very high doses of saccharin will not occur at the much lower doses of saccharin encountered in the human diet. At the high concentrations fed to rats, saccharin forms an insoluble precipitate in the bladder that subsequently results in chronic irritation of bladder epithelium, enhanced cell proliferation, and ultimately bladder tumors (Cohen, 1998, 1999). In vitro studies have shown that precipitation of saccharin in human urine will not occur at the concentrations that could be obtained from even extraordinary consumption of this artificial sweetener. As noted above and shown in Fig. 2-8, even for mutagenic chemicals that form DNA adducts, the response at high doses, as seen for DBC, may not be linear at low doses, although for another DNA-reactive carcinogen, AFB_1, the high-dose data were reflective of low-dose response in an approximately linear fashion. Examples such as these illustrate the importance of considering the molecular, biochemical, and cellular mechanisms responsible for toxicological responses when extrapolating from high to low dose and across species.

Toxicity tests are not designed to demonstrate that a chemical is safe but to characterize the toxic effects a chemical can produce. Although there are no set toxicology tests that have to be performed on every chemical intended for commerce, a tiered approach typical of many hazard assessment programs is illustrated in Fig. 2-13. Depending on the eventual use of the chemical, the toxic effects produced by structural analogs of the chemical, as well as the toxic effects produced by the chemical itself, contribute to the determination of the toxicology tests that should be performed. The FDA, EPA, and Organization for Economic Cooperation and Development (OECD) have written good laboratory practice (GLP) standards and other guidance that stipulate that procedure must be defined and accountability documented. These guidelines are expected to be followed when toxicity tests are conducted in support of the introduction of a chemical to the market.

The following sections provide an overview of basic toxicity testing procedures in use today. For a detailed description of these tests, the reader is referred to several authoritative texts on this subject (Barile, 2010; Hayes, 2008; Jacobson-Kram and Keller, 2006; Eaton and Gallagher, 2010).

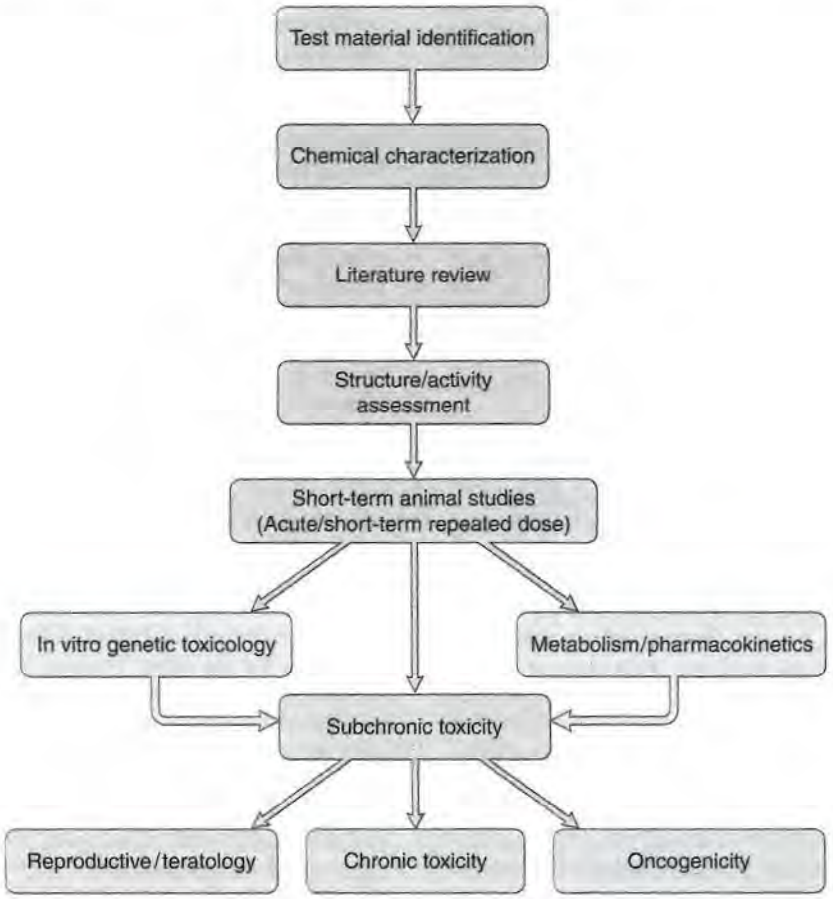

Figure 2-13. *Typical tiered testing scheme for the toxicological evaluation of new chemicals.* (From Wilson *et al.* 2008, Fig. 19-1, p. 918.)

Table 2-3

International Conference on Harmonization (ICH) Codification of "Safety" Protocols

Carcinogenicity studies	
S1A	Need for Carcinogenicity Studies of Pharmaceuticals
S1B	Testing for Carcinogenicity of Pharmaceuticals
S1C(R1)	Dose Selection for Carcinogenicity Studies of Pharmaceuticals & Limit Dose
Genotoxicity studies	
S2A	Guidance on Specific Aspects of Regulatory Genotoxicity Tests for Pharmaceuticals
S2B	Genotoxicity: A Standard Battery for Genotoxicity Testing of Pharmaceuticals
Toxicokinetics and pharmacokinetics	
S3A	Note for Guidance on Toxicokinetics: The Assessment of Systemic Exposure in Toxicity Studies
S3B	Pharmacokinetics: Guidance for Repeated Dose Tissue Distribution Studies
Toxicity testing	
	Single Dose Toxicity Tests
S4	Duration of Chronic Toxicity Testing in Animals (Rodent and Non Rodent Toxicity Testing)
Reproductive toxicology	
S5(R2)	Detection of Toxicity to Reproduction for Medicinal Products & Toxicity to Male Fertility
Biotechnological products	
S6	Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals
Pharmacology studies	
S7A	Safety Pharmacology Studies for Human Pharmaceuticals
S7B	The Non-Clinical Evaluation of the Potential for Delayed Ventricular Repolarization (QT Interval Prolongation) by Human Pharmaceuticals
Immunotoxicology studies	
S8	Immunotoxicity Studies for Human Pharmaceuticals
Joint safety/efficacy (multidisciplinary) topic	
M3(R1)	Non-Clinical Safety Studies for the Conduct of Human Clinical Trials for Pharmaceuticals

Titles and abbreviations adopted in November 2005. Data from http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Guidelines_Index.pdf.

Although different countries have often had different testing requirements for toxicity testing/product safety evaluation, efforts to "harmonize" such testing protocols have resulted in more standardized approaches. The International Conference on Harmonization (ICH) of Technical Requirements for Registration of Pharmaceuticals for Human Use includes regulatory authorities from Europe, Japan, and the United States (primarily the FDA), as well as experts from the pharmaceutical industry in the 3 regions, who worked together to develop internationally recognized scientific and technical approaches to pharmaceutical product registration. ICH has adopted guidelines for most areas of toxicity testing (Table 2-3). In addition to safety assessment (ICH guidelines designated with an "S"), ICH has also established guidelines on quality (Q), efficacy (E), and multidisciplinary (M) topics. (See http://www.ich.org/products/guidelines.html for a description of current ICH guidelines and reviews by Pugsley *et al.* (2008, 2011) for a detailed discussion of in vitro and in vivo approaches to safety pharmacology that has been informed by the ICH regulatory guidance document for preclinical safety testing of drugs.)

Typically, a tiered approach is used, with subsequent tests dependent on results of initial studies. A general framework for how new chemicals are evaluated for toxicity is shown in Fig 2-13. Early studies require careful chemical evaluation of the compound or mixture to assess purity, stability, solubility, and other physicochemical factors that could impact the ability of the test compound to be delivered effectively to animals. Once this information is obtained, the chemical structure of the test compound is compared with similar chemicals for which toxicological information is already available. Structure–activity relationships may be derived from a review of existing toxicological literature, and can provide additional guidance on design of acute and repeated-dose experiments, and what specialized tests need to be completed. Once such basic information has been compiled and evaluated, the test compound is then administered to animals in acute and repeated-dose studies.

Because of increased societal pressure to reduce or eliminate the use of animals in toxicity testing, while also ensuring that new chemicals do not represent unreasonable risks to human health or the environment, regulatory agencies have been encouraging new approaches to descriptive toxicity tests that do not rely on laboratory animals. For example, the European Union (EU) promulgated an important regulatory initiative for the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH). The implementation of REACH "will have significant impact on applied toxicology and exposure assessment by stimulating innovation in sampling and analysis, toxicology testing, exposure modeling, alternative toxicity testing, and risk assessment practices."

(Williams *et al.*, 2009b). Alternative, in vitro approaches to toxicity assessment are likely to transform the way that product safety evaluation is done in the future, although the standard approaches to hazard evaluation described in this section are likely to continue as the mainstay of toxicity evaluation for the next decade, irrespective of the fact that some areas, such as acute toxicity testing and eye irritation, are likely to be largely replaced by in vitro tests in the next decade (Ukelis *et al.*, 2008).

The development of new "omics" technologies (discussed later in this section) may have profound implications for toxicity testing in the future (NAS/NRC, 2007). The recognition that many of the existing chemicals in commercial use today, as well as new chemicals being introduced into commerce, have little toxicological information about them has prompted calls for new "high-throughput" approaches to toxicity testing that will allow at least basic hazard characterization for the thousands of untested chemicals currently in the marketplace, as well as the many new chemicals that are introduced each year. A report from the National Academy of Sciences/National Research Council in 2007 called for a "paradigm shift" in how toxicity testing is done (NAS/NRC, 2007). A key component of this new vision on toxicity testing is the use of an extensive battery of in vitro tests to evaluate "pathways" of toxicity (NAS/NRC, 2010). The hope is that new technologies in genomics, transcriptomics, proteomics, metabolomics, and bioinformatics (discussed later in this chapter) can be combined with automated high-throughput technologies to create a tiered structure for toxicity testing. The approach to using biochemical and molecular pathway-based analyses, rather than apical end points (eg, target organ damage, mutagenesis, carcinogenesis, reproductive and developmental effects), to identify potentially problematic chemicals early in their development is particularly attractive from a time frame and economic perspective (NAS/NRC, 2010). However, it is also recognized that validation of such tests is critically important to the reliable use of such screening technologies, and that the traditional in vivo studies described in the following section will continue to serve an important role in hazard evaluations for years to come, especially as a means of validating new high-throughput screening approaches.

Acute Toxicity Testing

Generally, the first toxicity test performed on a new chemical is acute toxicity, determined from the administration of a single exposure. The objectives of acute toxicity testing are to: (1) provide an estimate of the intrinsic toxicity of the substance, often times expressed as an approximate LD (eg, LD_{50}), (2) provide information on target organs and other clinical manifestations of toxicity, (3) identify species differences and susceptible species, (4) establish the reversibility of the toxic response, and (5) provide information that will assist in the design and dose selection for longer-term (subchronic, chronic) studies. It should be noted that the ICH recommended in 1991 (D'Arcy and Harron, 1992) the elimination of LD_{50} determinations for pharmaceuticals, although other regulatory requirements, for example, pesticide registration, may still require determinations of LD_{50}s.

The LD_{50} and other acute toxic effects are determined after 1 or more routes of administration (1 route being oral or the intended route of exposure) in 1 or more species. The species most often used are the mouse and rat. Studies are performed in both adult male and female animals. Food is often withheld the night before dosing. The number of animals that die in a 14-day period after a single dosage is tabulated. In addition to mortality and weight, daily examination of test animals should be conducted for signs of intoxication, lethargy, behavioral modifications, morbidity, food consumption, and so on.

Determination of the LD_{50} has become a public issue because of increasing concern for the welfare and protection of laboratory animals. The LD_{50} is not a biological constant. Many factors influence toxicity and thus may alter the estimation of the LD_{50} in any particular study. Factors such as animal strain, age, and weight, type of feed, caging, pretrial fasting time, method of administration, volume and type of suspension medium, and duration of observation have all been shown to influence adverse responses to toxic substances. These and other factors have been discussed in detail in earlier editions of this textbook (Doull, 1980). Because of this inherent variability in LD_{50} estimates, it is now recognized that for most purposes it is only necessary to characterize the LD_{50} within an order of magnitude range such as 5 to 50 mg/kg, 50 to 500 mg/kg, and so on.

There are several traditional approaches to determining the LD_{50} and its 95% confidence limit as well as the slope of the probit line. The reader is referred to the classic works of Litchfield and Wilcoxon (1949), Bliss (1957), and Finney (1971) for a description of the mechanics of these procedures. Other statistical techniques that require fewer animals, such as the "moving averages" method of Thompson and Weill (Weil, 1952), are available but do not provide confidence limits for the LD_{50} and the slope of the probit line. Finney (1985) has succinctly summarized the advantages and deficiencies of many of the traditional methods. For most circumstances, an adequate estimate of the LD_{50} and an approximation of the 95% confidence intervals can be obtained with as few as 6 to 9 animals, using the "up-and-down" method as modified by Bruce (1985). When this method was compared with traditional methods that typically utilize 40 to 50 animals, excellent agreement was obtained for all 10 compounds tested (Bruce, 1987). In mice and rats the LD_{50} is usually determined as described above, but in the larger species only an approximation of the LD_{50} is obtained by increasing the dose in the same animal until serious toxic effects are evident.

Alternative in vitro approaches to estimating the LD_{50} have been proposed. For example, the *Registry of Cytotoxicity* (RC), originally published in German in 1998 (Halle, 2003), was developed by linear regression analysis of the mean IC_{50} values determined in mammalian cells in culture and the LD_{50} values reported in the literature from various laboratory species. Using this approach, the authors predicted (within a reasonable dose range) the acute oral LD_{50} for 252 of 347 xenobiotics, and the intravenous LD_{50} for rats and/or mice for 117 of 150 xenobiotics (Halle, 2003). Of course, such in vitro approaches do not fully account for dispositional effects that could result in large species differences in acute toxicity, but do provide a rapid first approximation of acute toxicity without the use of experimental animals.

If there is a reasonable likelihood of substantial exposure to the material by dermal or inhalation exposure, acute dermal and acute inhalation studies are performed. When animals are exposed acutely to chemicals in the air they breathe or the water they (fish) live in, the dose the animals receive is usually not known. For these situations, the lethal concentration 50 (LC_{50}) is usually determined, that is, the concentration of chemical in the air or water that causes death to 50% of the animals. In reporting an LC_{50}, it is imperative that the time of exposure be indicated. The acute dermal toxicity test is usually performed in rabbits. The site of application is shaved. The test substance is kept in contact with the skin for 24 hours by wrapping the skin with an impervious plastic material. At the end of the exposure period, the wrapping is removed and the skin is wiped to remove any test substance still remaining. Animals are observed at various intervals for 14 days, and the LD_{50} is calculated. If no

toxicity is evident at 2 g/kg, further acute dermal toxicity testing is usually not performed. Acute inhalation studies are performed that are similar to other acute toxicity studies except that the route of exposure is inhalation. Most often, the length of exposure is 4 hours.

By themselves LD_{50} and LC_{50} values are of limited significance given the growing sophistication of target organ toxicity end points and mechanistic analysis. The most meaningful scientific information derived from acute toxicity tests comes from clinical observations and post-mortem examination of animals rather than from the specific LD_{50} value.

Skin and Eye Irritations

The ability of a chemical to irritate the skin and eye after an acute exposure is usually determined in rabbits. For the dermal irritation test (Draize test), rabbits are prepared by removal of fur on a section of the back by electric clippers. The chemical is applied to the skin (0.5 mL of liquid or 0.5 g of solid) under 4 covered gauze patches (1 in square; 1 intact and 2 abraded skin sites on each animal) and usually kept in contact for 4 hours. The nature of the covering patches depends on whether occlusive, semiocclusive, or nonocclusive tests are desired. For occlusive testing, the test material is covered with an impervious plastic sheet; for semiocclusive tests, a gauze dressing may be used. Occasionally, studies may require that the material be applied to abraded skin. The degree of skin irritation is scored for erythema (redness), eschar (scab), and edema (swelling) formation, and corrosive action. These dermal irritation observations are repeated at various intervals after the covered patch has been removed. To determine the degree of ocular irritation, the chemical is instilled into 1 eye (0.1 mL of liquid or 100 mg of solid) of each test rabbit. The contralateral eye is used as the control. The eyes of the rabbits are then examined at various times after application.

Controversy over this test has led to the development of alternative in vitro models for evaluating cutaneous and ocular toxicity of substances. The various in vitro methods that have been evaluated for this purpose include epidermal keratinocyte and corneal epithelial cell culture models. Several commercially available "reconstructed human epidermis" models have been developed explicitly for the purposes of in vitro skin irritation and corrosion tests (Netzlaff *et al.*, 2005).

Sensitization

Information about the potential of a chemical to sensitize skin is needed in addition to irritation testing for all materials that may repeatedly come into contact with the skin. Numerous procedures have been developed to determine the potential of substances to induce a sensitization reaction in humans (delayed hypersensitivity reaction), including the Draize test, the open epicutaneous test, the Buehler test, Freund's complete adjuvant test, the optimization test, the split adjuvant test, and the guinea pig maximization test (Hayes *et al.*, 2008; Rush *et al.*, 1995). Although they differ in regard to route and frequency of duration, they all utilize the guinea pig as the preferred test species. In general, the test chemical is administered to the shaved skin topically, intradermally, or both and may include the use of adjuvant to enhance the sensitivity of the assay. Multiple administrations of the test substance are generally given over a period of 2 to 4 weeks. Depending on the specific protocol, the treated area may be occluded. Approximately 2 to 3 weeks after the last treatment, the animals are challenged with a nonirritating concentration of the test substance and the development of erythema is evaluated.

Subacute (Repeated-Dose Study)

Subacute toxicity tests are performed to obtain information on the toxicity of a chemical after repeated administration and as an aid to establish doses for subchronic studies. A typical protocol is to give 3 to 4 different dosages of the chemicals to the animals by mixing it in their feed. For rats, 10 animals per sex per dose are often used; for dogs, 3 dosages and 3 to 4 animals per sex are used. Clinical chemistry and histopathology are performed after either 14 or 28 days of exposure, as described in the section "Subchronic."

Subchronic

The toxicity of a chemical after subchronic exposure is then determined. Subchronic exposure can last for different periods of time, but 90 days is the most common test duration. The principal goals of the subchronic study are to establish a NOAEL and to further identify and characterize the specific organ or organs affected by the test compound after repeated administration. One may also obtain a "lowest observed adverse effect level" (LOAEL) as well as the NOAEL for the species tested. The numbers obtained for NOAEL and LOAEL will depend on how closely the dosages are spaced and the number of animals examined. Determinations of NOAELs and LOAELs have numerous regulatory implications. For example, the EPA utilizes the NOAEL to calculate the *reference dose* (RfD), which may be used to establish regulatory values for "acceptable" pollutant levels (Barnes and Dourson, 1988) (see Chap. 4). An alternative to the NOAEL approach referred to as the *benchmark dose* uses all the experimental data to fit 1 or more dose–response curves (Crump, 1984). These curves are then used to estimate a benchmark dose that is defined as "the statistical lower bound on a dose corresponding to a specified level of risk" (Allen *et al.*, 1994a). Although subchronic studies are frequently the primary or sole source of experimental data to determine both the NOAEL and the benchmark dose, these concepts can be applied to other types of toxicity testing protocols, such as that for chronic toxicity or developmental toxicity (Allen *et al.*, 1994a,b; Faustman *et al.*, 1994) (see also Chap. 4 for a complete discussion of the derivation and use of NOAELs, RfDs, and benchmark doses). If chronic studies have been completed, these data are generally used for NOAEL and LOAEL estimates in preference to data from subchronic studies.

A subchronic study is usually conducted in 2 species (usually rat and dog for FDA, and mouse for EPA) by the route of intended exposure (usually oral). At least 3 doses are employed (a high dose that produces toxicity but does not cause more than 10% fatalities, a low dose that produces no apparent toxic effects, and an intermediate dose) with 10 to 20 rodents and 4 to 6 dogs of each sex per dose. Each animal should be uniquely identified with permanent markings such as ear tags, tattoos, or electronically coded microchip implants. Only healthy animals should be used, and each animal should be housed individually in an adequately controlled environment. When the test compound is administered in the diet over a prolonged period of time (subchronic and chronic studies), the concentration in the diet should be adjusted periodically (weekly for the first 12–14 weeks) to maintain a constant intake of material based on food consumption and rate of change in body weight (Wilson *et al.*, 2008). Animals should be observed once or twice daily for signs of toxicity, including changes in body weight, diet consumption, changes in fur color or texture, respiratory or cardiovascular distress, motor and behavioral abnormalities, and palpable masses. All premature deaths should be recorded and necropsied as soon as possible. Severely moribund animals should be terminated immediately to preserve tissues and reduce unnecessary suffering.

At the end of the 90-day study, all the remaining animals should be terminated and blood and tissues should be collected for further analysis. The gross and microscopic condition of the organs and tissues (about 15–20) and the weight of the major organs (about 12) are recorded and evaluated. Hematology and blood chemistry measurements are usually done before, in the middle of, and at the termination of exposure. Hematology measurements usually include hemoglobin concentration, hematocrit, erythrocyte counts, total and differential leukocyte counts, platelet count, clotting time, and prothrombin time. Clinical chemistry determinations commonly made include glucose, calcium, potassium, urea nitrogen, ALT, serum AST, gamma-glutamyltranspeptidase (GGT), sorbitol dehydrogenase, lactic dehydrogenase, alkaline phosphatase, creatinine, bilirubin, triglycerides, cholesterol, albumin, globulin, and total protein. Urinalysis is usually performed in the middle of and at the termination of the testing period and often includes determination of specific gravity or osmolarity, pH, proteins, glucose, ketones, bilirubin, and urobilinogen as well as microscopic examination of formed elements. If humans are likely to have significant exposure to the chemical by dermal contact or inhalation, subchronic dermal and/or inhalation experiments may also be required. Subchronic toxicity studies not only characterize the dose–response relationship of a test substance after repeated administration but also provide data for a more reasonable prediction of appropriate doses for chronic exposure studies.

For chemicals that are to be registered as drugs, acute and subchronic studies (and potentially additional special tests if a chemical has unusual toxic effects or therapeutic purposes) must be completed before the company can file an Investigational New Drug (IND) application with the FDA. If the application is approved, clinical trials can commence. At the same time phase I, phase II, and phase III clinical trials are performed, chronic exposure of the animals to the test compound can be carried out in laboratory animals, along with additional specialized tests.

Chronic

Long-term or chronic exposure studies are performed similarly to subchronic studies except that the period of exposure is longer than 3 months. In rodents, chronic exposures are usually for 6 months to 2 years. Chronic studies in nonrodent species are usually for 1 year but may be longer. The length of exposure is somewhat dependent on the intended period of exposure in humans. For example, for pharmaceuticals, the ICH S4 guidance calls for studies of 6 months in duration in rodents, and 9 months in nonrodents. However, if the chemical is a food additive with the potential for lifetime exposure in humans, a chronic study up to 2 years in duration is likely to be required.

Dose selection is critical in these studies to ensure that premature mortality from chronic toxicity does not limit the number of animals that survive to a normal life expectancy. Most regulatory guidelines require that the highest dose administered be the estimated maximum tolerable dose (MTD, also commonly referred to as the "minimally toxic dose"). This is generally derived from subchronic studies, but additional longer studies (eg, 6 months) may be necessary if delayed effects or extensive cumulative toxicity are indicated in the 90-day subchronic study. The MTD has had various definitions (Haseman, 1985). It has been defined by some regulatory agencies as the dose that suppresses body weight gain slightly (ie, 10%) in a 90-day subchronic study (Reno, 1997). However, regulatory agencies may also consider the use of parameters other than weight gain, such as physiological and pharmacokinetic considerations and urinary metabolite profiles, as indicators of an appropriate MTD (Reno, 1997). Generally, 1 or 2 additional doses, usually fractions of the MTD (eg, one-half and one-quarter MTD), and a control group are tested.

Chronic toxicity tests may include a consideration of the carcinogenic potential of chemicals so that a separate lifetime feeding study that addresses carcinogenicity does not have to be performed. However, specific chronic studies designed to assess the carcinogenic potential of a substance may be required (see below).

Developmental and Reproductive Toxicity

The effects of chemicals on reproduction and development also need to be determined. *Developmental toxicology* is the study of adverse effects on the developing organism occurring anytime during the life span of the organism that may result from exposure to chemical or physical agents before conception (either parent), during prenatal development, or postnatally until the time of puberty. *Teratology* is the study of defects induced during development between conception and birth (see Chap. 10). *Reproductive toxicology* is the study of the occurrence of adverse effects on the male or female reproductive system that may result from exposure to chemical or physical agents (see Chap. 20).

Several types of animal tests are utilized to examine the potential of an agent to alter development and reproduction. (For a detailed description of reproductive and developmental toxicity testing procedures, see Christian [2008].) General fertility and reproductive performance (segment I) tests are usually performed in rats with 2 or 3 doses (20 rats per sex per dose) of the test chemical (neither produces maternal toxicity). Males are given the chemical 60 days and females 14 days before mating. The animals are given the chemical throughout gestation and lactation. Typical observations made include the percentage of females that become pregnant, the number of stillborn and live offspring, and the weight, growth, survival, and general condition of the offspring during the first 3 weeks of life.

The potential of chemicals to disrupt normal embryonic and/or fetal development (teratogenic effects) is also determined in laboratory animals. Current guidelines for these segment II studies call for the use of 2 species, including 1 nonrodent species (usually rabbits). Teratogens are most effective when administered during the first trimester, the period of organogenesis. Thus, the animals (usually 12 rabbits and 24 rats or mice per group) are usually exposed to 1 of 3 dosages during organogenesis (days 7-17 in rodents and 7-19 in rabbits), and the fetuses are removed by cesarean section a day before the estimated time of delivery (gestational days 29 for rabbit, 20 for rat, and 18 for mouse). The uterus is excised and weighed and then examined for the number of live, dead, and resorbed fetuses. Live fetuses are weighed; half of each litter is examined for skeletal abnormalities and the remaining half for soft tissue anomalies.

The perinatal and postnatal toxicities of chemicals also are often examined (segment III). This test is performed by administering the test compound to rats from the 15th day of gestation throughout delivery and lactation and determining its effect on the birth weight, survival, and growth of the offspring during the first 3 weeks of life.

In some instances a multigenerational study may be chosen, often in place of segment III studies, to determine the effects of chemicals on the reproductive system. At least 3 dosage levels are given to groups of 25 female and 25 male rats shortly after weaning (30–40 days of age). These rats are referred to as the F_0 generation. Dosing continues throughout breeding (about 140 days of age), gestation, and lactation. The offspring (F_1 generation) have

thus been exposed to the chemical in utero, via lactation, and in the feed thereafter. When the F_1 generation is about 140 days old, about 25 females and 25 males are bred to produce the F_2 generation, and administration of the chemical is continued. The F_2 generation is thus also exposed to the chemical in utero and via lactation. The F_1 and F_2 litters are examined as soon as possible after delivery. The percentage of F_0 and F_1 females that get pregnant, the number of pregnancies that go to full term, the litter size, the number of stillborn, and the number of live births are recorded. Viability counts and pup weights are recorded at birth and at 4, 7, 14, and 21 days of age. The fertility index (percentage of mating resulting in pregnancy), gestation index (percentage of pregnancies resulting in live litters), viability index (percentage of animals that survive 4 days or longer), and lactation index (percentage of animals alive at 4 days that survived the 21-day lactation period) are then calculated. Gross necropsy and histopathology are performed on some of the parents (F_0 and F_1), with the greatest attention being paid to the reproductive organs, and gross necropsy is performed on all weanlings.

The International Conference on Harmonization (ICH) guidelines provide for flexible guidelines that address 6 "ICH stages" of development: premating and conception (stage A), conception to implantation (stage B), implantation to closure of the hard palate (stage C), closure of the hard palate to end of pregnancy (stage D), birth and weaning (stage E), and weaning to sexual maturity (stage F). All of these stages are covered in the segment I to segment III studies described above (Christian, 2008).

Numerous short-term tests for teratogenicity have been developed (Faustman, 1988). These tests utilize whole-embryo culture, organ culture, and primary and established cell cultures to examine developmental processes and estimate the potential teratogenic risks of chemicals. Many of these in utero test systems are under evaluation for use in screening new chemicals for teratogenic effects. These systems vary in their ability to identify specific teratogenic events and alterations in cell growth and differentiation. In general, the available assays cannot identify functional or behavioral teratogens (Faustman, 1988).

Mutagenicity

Mutagenesis is the ability of chemicals to cause changes in the genetic material in the nucleus of cells in ways that allow the changes to be transmitted during cell division. Mutations can occur in either of 2 cell types, with substantially different consequences. Germinal mutations damage DNA in sperm and ova, which can undergo meiotic division and therefore have the potential for transmission of the mutations to future generations. If mutations are present at the time of fertilization in either the egg or the sperm, the resulting combination of genetic material may not be viable, and the death may occur in the early stages of embryonic cell division. Alternatively, the mutation in the genetic material may not affect early embryogenesis but may result in the death of the fetus at a later developmental period, resulting in abortion. Congenital abnormalities may also result from mutations. Somatic mutations refer to mutations in all other cell types and are not heritable but may result in cell death or transmission of a genetic defect to other cells in the same tissue through mitotic division. Because the initiating event of chemical carcinogenesis is thought to be a mutagenic one, mutagenic tests are often used to screen for potential carcinogens.

Numerous in vivo and in vitro procedures have been devised to test chemicals for their ability to cause mutations. Some genetic alterations are visible with the light microscope. In this case, cytogenetic analysis of bone marrow smears is used after the animals have been exposed to the test agent. Because some mutations are incompatible with normal development, the mutagenic potential of a chemical can also be evaluated by the dominant lethal test. This test is usually performed in rodents. The male is exposed to a single dose of the test compound and then is mated with 2 untreated females weekly for 8 weeks. The females are killed before term, and the number of live embryos and the number of corpora lutea are determined.

The test for mutagens that has received the widest attention is the *Salmonella*/microsome test developed by Ames *et al.* (1975). This test uses several mutant strains of *Salmonella typhimurium* that lack the enzyme phosphoribosyl ATP synthetase, which is required for histidine synthesis. These strains are unable to grow in a histidine-deficient medium unless a reverse or back mutation to the wild type has occurred. Other mutations in these bacteria have been introduced to enhance the sensitivity of the strains to mutagenesis. The 2 most significant additional mutations enhance penetration of substances into the bacteria and decrease the ability of the bacteria to repair DNA damage. Because many chemicals are not mutagenic or carcinogenic unless they are biotransformed to a toxic product by enzymes in the endoplasmic reticulum (microsomes), rat liver microsomes are usually added to the medium containing the mutant strain and the test chemical. The number of reverse mutations is then quantified by the number of bacterial colonies that grow in a histidine-deficient medium.

Strains of yeast have recently been developed that detect genetic alterations arising during cell division after exposure to nongenotoxic carcinogens as well as mutations that arise directly from genotoxic carcinogens. This test identifies deletions of genetic material that occur during recombination events in cell division that may result from oxidative damage to DNA, direct mutagenic effects, alterations in fidelity of DNA repair, and/or changes in cell cycle regulation (Galli and Schiestl, 1999). Mutagenicity is discussed in detail in Chap. 9.

With the advent of techniques that readily allow manipulation of the mouse genome, transgenic animals have been developed that allow for in vivo assessment of mutagenicity of compounds. For example, 2 commercially available mouse strains, the "MutaMouse" and "Big Blue," contain the *lac* operon of *E. coli* that has been inserted into genomic DNA using a lambda phage to DNA to produce a recoverable shuttle vector. Stable, homozygous strains of these transgenic animals (both mice and rats have been engineered) can be exposed to potential mutagenic agents. Following in vivo exposure, the target *lac* genes can be recovered from virtually any cell type or organ and analyzed for mutations (Brusick *et al.*, 2008).

Oncogenicity Bioassays

Oncogenicity studies are both time consuming and expensive, and are usually only done when there is reason to suspect that a chemical may be carcinogenic, or when there may be wide spread, long-term exposures to humans (eg, widely used food additives, drinking water contaminants, or pharmaceuticals that are likely to be administered repeatedly for long periods of time). Chemicals that test positive in several mutagenicity assays are likely to be carcinogenic, and thus are frequent candidates for oncogenicity bioassay assessment. In the United States, the National Toxicology Program (NTP) has the primary responsibility for evaluating nondrug chemicals for carcinogenic potential. For pharmaceuticals, the FDA may require the manufacturer to conduct oncogenicity studies as part of the preclinical assessment, depending on the intended use of the drug, and the results of mutagenicity assays and other toxicological data.

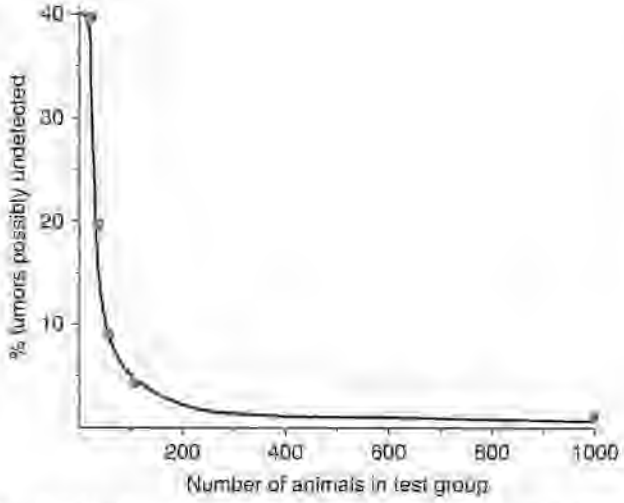

Figure 2-14. Statistical limitations in the power of experimental animal studies to detect tumorigenic effects.

Studies to evaluate the oncogenic (carcinogenic) potential of chemicals are usually performed in rats and mice and extend over the average lifetime of the species (18 months to 2 years for mice, 2–2.5 years for rats). To ensure that 30 rats per dose survive the 2-year study, 60 rats per group per sex are often started in the study. Both gross and microscopic pathological examinations are made not only on animals that survive the chronic exposure but also on those that die prematurely. The use of the MTD in carcinogenicity has been the subject of controversy. The premise that high doses are necessary for testing the carcinogenic potential of chemicals is derived from the statistical and experimental design limitations of chronic bioassays. Consider that a 0.5% increase in cancer incidence in the United States would result in over 1 million additional cancer deaths each year—clearly an unacceptably high risk. However, identifying with statistical confidence a 0.5% incidence of cancer in a group of experimental animals would require a minimum of 1000 test animals, and this assumes that no tumors were present in the absence of exposure (zero background incidence).

Fig. 2-14 shows the statistical relationship between minimum detectable tumor incidence and the number of test animals per group. This curve shows that in a chronic bioassay with 50 animals per test group, a tumor incidence of about 8% could exist even though no animals in the test group had tumors. This example assumes that there are no tumors in the control group. These statistical considerations illustrate why animals are tested at doses higher than those that occur in human exposure. Because it is impractical to use the large number of animals that would be required to test the potential carcinogenicity of a chemical at the doses usually encountered by people, the alternative is to assume that there is a relationship between the administered dose and the tumorigenic response and give animals doses of the chemical that are high enough to produce a measurable tumor response in a reasonable size test group, such as 40 to 50 animals per dose. The limitations of this approach are discussed in Chap. 4. For nonmutagenic pharmaceutical agents, ICH S1C provides the following guidance on dose selection for oncogenicity studies: "The doses selected for rodent bioassays for nongenotoxic pharmaceuticals should provide an exposure to the agent that (1) allow an adequate margin of safety over the human therapeutic exposure, (2) are tolerated without significant chronic physiological dysfunction and are compatible with good survival, (3) are guided by a comprehensive set of animal and human data that focus broadly on the properties of the agent and the suitability of the animal (4) and permit data interpretation in the context of clinical use."

Another approach for establishing maximum doses for use in chronic animal toxicity testing of drugs is often used for substances for which basic human pharmacokinetic data are available (eg, new pharmaceutical agents that have completed phase I clinical trials). For chronic animal studies performed on drugs where single-dose human pharmacokinetic data are available, a daily dose that would provide an area under the curve (AUC) in laboratory animals equivalent to 25 times the AUC in humans given the highest (single) daily dose to be used therapeutically may be used, rather than the MTD. Based on a series of assumptions regarding allometric scaling between rodents and humans (Table 2-2), the ICH noted that it may not be necessary to exceed a dose of 1500 mg/kg per day where there is no evidence of genotoxicity, and where the maximum recommended human dose does not exceed 500 mg per day.

Most regulatory guidelines require that both benign and malignant tumors be reported in oncogenicity bioassays. Statistical increases above the control incidence of tumors (either all tumors or specific tumor types) in the treatment groups are considered indicative of carcinogenic potential of the chemical unless there are qualifying factors that suggest otherwise (lack of a dose response, unusually low incidence of tumors in the control group compared with "historic" controls, etc; Huff, 1999). Thus, the conclusion as to whether a given chronic bioassay is positive or negative for carcinogenic potential of the test substance requires careful consideration of background tumor incidence. Properly designed chronic oncogenicity studies require that a concurrent control group matched for variables such as age, diet, and housing conditions be used. For some tumor types, the "background" incidence of tumors is surprisingly high. Fig. 2-15 shows the background tumor incidence for

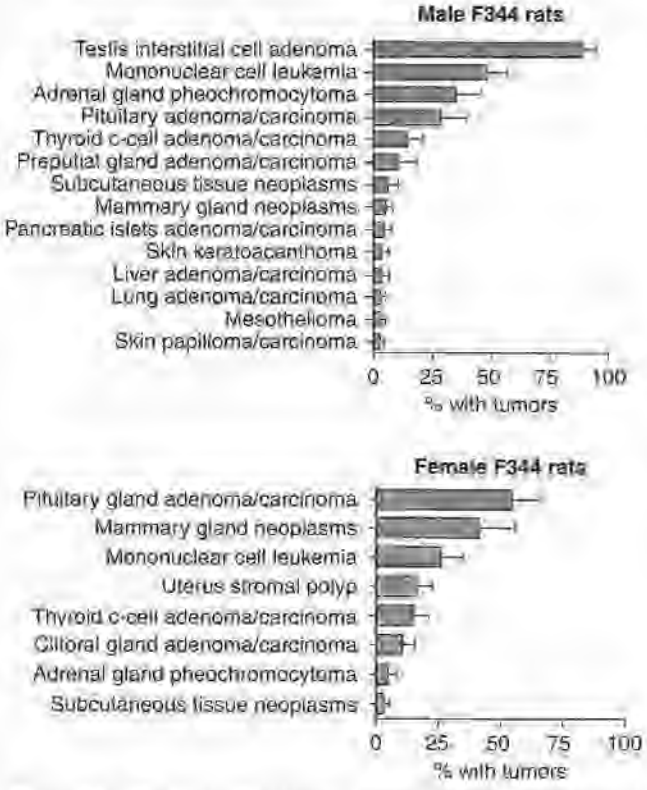

Figure 2-15. Most frequently occurring tumors in untreated control rats from recent NTP 2-year rodent carcinogenicity studies. The values shown represent the mean ± SD of the percentage of animals developing the specified tumor type at the end of the 2-year study. The values were obtained from 27 different studies involving a combined total of between 1519 and 1553 animals per tumor type.

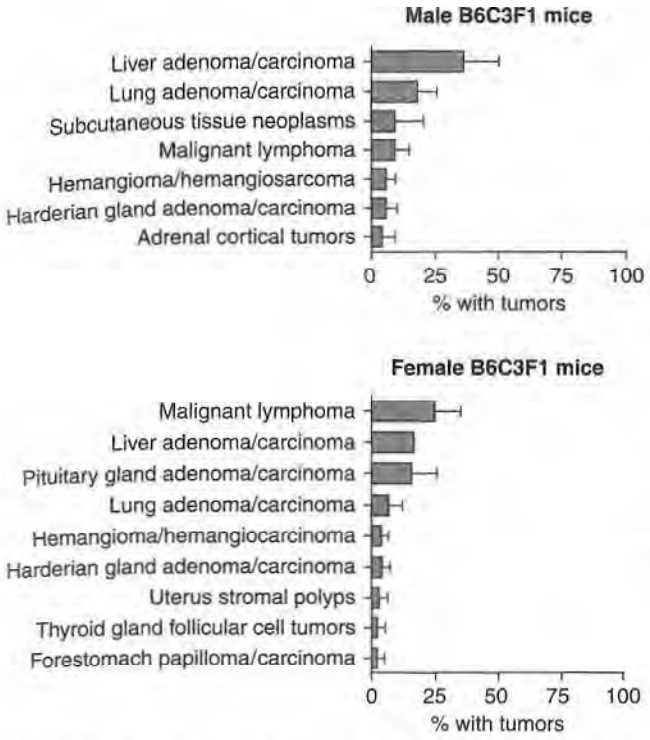

Figure 2-16. *Most frequently occurring tumors in untreated control mice from recent NTP 2-year rodent carcinogenicity studies.* The values shown represent the mean ± SD of the percentage of animals developing the specified tumor type at the end of the 2-year study. The values were obtained from 30 different studies involving a total of between 1447 and 1474 animals per tumor type.

various tumors in male and female F344 rats used in 27 NTP 2-year rodent carcinogenicity studies. The data shown represent the percent of animals in control (nonexposed) groups that developed the specified tumor type by the end of the 2-year study. These studies involved more than 1300 rats of each sex. Fig. 2-16 shows similar data for control (nonexposed) male and female B6C3F1 mice from 30 recent NTP 2-year carcinogenicity studies and includes data from over 1400 mice of each sex. There are several key points that can be derived from these summary data:

1. Tumors, both benign and malignant, are not uncommon events in animals even in the absence of exposure to any known carcinogen.
2. There are numerous different tumor types that develop "spontaneously" in both sexes of both rats and mice, but at different rates.
3. Background tumors that are common in 1 species may be uncommon in another (eg, testicular interstitial cell adenomas are very common in male rats but rare in male mice; liver adenomas/carcinomas are about 10 times more prevalent in male mice than in male rats).
4. Even within the same species and strain, large gender differences in background tumor incidence are sometimes observed (eg, adrenal gland pheochromocytomas are about 7 times more prevalent in male F344 rats than in female F344 rats; lung and liver tumors are twice as prevalent in male B6C3F1 mice as in female B6C3F1 mice).
5. Even when the general protocols, diets, environment, strain and source of animals, and other variables are relatively constant, background tumor incidence can vary widely, as shown by the relatively large SDs for some tumor types in the NTP bioassay program. For example, the range in liver adenoma/carcinoma incidence in 30 different groups of unexposed (control) male B6C3F1 mice went from a low of 10% to a high of 68%. Pituitary gland adenomas/carcinomas ranged from 12% to 60% and 30% to 76% in unexposed male and female F344 rats, respectively, and from 0% to 36% in unexposed female B6C3F1 mice.

Taken together, these data demonstrate the importance of including concurrent control animals in such studies. In addition, comparisons of the concurrent control results to "historic" controls accumulated over years of study may be important in identifying potentially spurious "false-positive" results. The relatively high variability in background tumor incidence among groups of healthy, highly inbred strains of animals maintained on nutritionally balanced and consistent diets in rather sterile environments highlights the dilemma in interpreting the significance of both positive and negative results in regard to the human population, which is genetically diverse, has tremendous variability in diet, nutritional status, and overall health, and lives in an environment full of potentially carcinogenic substances, both natural and human-made.

Finally, it should be noted that both inbred and outbred strains have distinct background tumor patterns and the NTP and most other testing programs select strains based on the particular needs of the agent under study. For example, the NTP used the Wistar rat for chemicals that may have the testis as a target organ, based on acute, subchronic, or other bioassay results. Similarly, the NTP used the Sprague–Dawley strain of rat in studies of estrogenic agents such as genistein because its mammary tumors are responsive to estrogenic stimulation, as are humans'.

Neurotoxicity Assessment

Neurotoxicity or a neurotoxic effect is defined as an adverse change in the chemistry, structure, or function of the nervous system following exposure to a chemical or physical agent. The structure, function, and development of the nervous system and its vulnerability to chemicals are examined in Chap. 16. When evaluating the potential neurological effects of a compound, effects may be on the central or peripheral nervous system or related to exposure that occurred during development or as an adult. The developing nervous system is particularly sensitive to chemical exposures (see Chap. 10).

In vitro systems often using cell culture techniques are a rapidly developing area of neurotoxicity assessment. Specific cell lines are available to examine effects on neuron or glial cells such as proliferation, migration, apoptosis, synaptogenesis, and other end points. In vitro assays have a number of potential advantages including minimizing the use of animal, lower costs, and adaptable to high-throughput screening. It is also possible to use an in vitro model to examine the interaction of chemicals, such as food additives, on neuronal cells (Lau *et al.*, 2006). The principles and challenges of in vitro neurotoxicity testing are well described (Claudio, 1992; Tiffany-Castiglioni, 2004).

Procedures for the neurobehavioral evaluation of animals were initially developed as part of the scientific investigation of behavioral motivation. Some of these procedures were then used to evaluate the neuropharmacological properties of new drugs. Now animals are commonly used to evaluate the neurotoxic properties of chemicals. A wide range of adult and developmental animal tests are used to access neurobehavioral function. In addition, neuropathological assessment is an important part of the neurotoxicity evaluation and

best practices have been developed for developmental neurotoxicity (DNT) (Bolon *et al.*, 2006). Irwin developed a basic screen for behavioral function in mice (Irwin, 1968), which was subsequently refined to the functional observational battery (FOB) (Moser, 2000). The FOB can also be used in the evaluation of drug safety (Redfern *et al.*, 2005).

The US EPA established a protocol for the evaluation of DNT in laboratory animals (US EPA 870.6300 and OECD 426) (EPA, 1998; OECD, 2004). These protocols include tests of neurobehavioral function, such as auditory startle, learning and memory function, changes in motor activity, and neuropathological examination and morphometric analysis. Methods and procedures for DNT evaluation are well established (Claudio *et al.*, 2000; Cory-Slechta *et al.*, 2001; Dorman *et al.*, 2001; Garman *et al.*, 2001; Mileson and Ferenc, 2001). Recent studies examine the neurotoxicity of multiple chemical exposures in animals (Moser *et al.*, 2006). Methods are also available to examine cognitive measures on weanling rodents in DNT studies (Ehman and Moser, 2006). Nonhuman primates have been invaluable in evaluating the effects of neurotoxicants and the risk assessment process (Burbacher and Grant, 2000). Sophisticated assessment of operant behavior, and learning and memory assessment of rodents, has been used to evaluate the effects of lead (Cory-Slechta, 1995, 1996, 2003). Monkeys can also be used to evaluate the low-level effects of neurotoxicants such as mercury on vision, auditory function, and vibration sensitivity (Burbacher *et al.*, 2005; Rice and Gilbert, 1982, 1992, 1995). There is remarkable concordance between human and animal neurotoxicity assessment, for example, in lead, mercury, and PCBs (Rice, 1995).

Human testing for the neurological effects of occupational exposures to chemicals (Anger, 2003; Farahat *et al.*, 2003; Kamel *et al.*, 2003; McCauley *et al.*, 2006), and even the neurotoxic effects of war (Binder *et al.*, 1999, 2001), is advancing rapidly. These methods have also been applied to Hispanic workers (Rohlman *et al.*, 2001b) and populations with limited education or literacy (Rohlman *et al.*, 2003). The WHO has also recommended a test battery for humans (Anger *et al.*, 2000). There are also neurobehavioral test batteries for assessing children (Rohlman *et al.*, 2001a). Evaluation of the childhood neurological effects of lead (Lanphear *et al.*, 2005; Needleman and Bellinger, 1991) and mercury (Myers *et al.*, 2000) has added enormously to our understanding of the health effects of these chemicals and to the methodology of human neurobehavioral testing.

In summary, the neurotoxicological evaluation is an important aspect of developing risk assessments for environmental chemicals and drugs.

Immunotoxicity Assessment

Under normal conditions, the immune system is responsible for host defense against pathogenic infections and certain cancers. However, environmental exposures can alter immune system development and/or function and lead to hypersensitivity, autoimmunity, or immunosuppression, the outcome of which may be expressed as a pathology in most any organ or tissue (see Chap. 12). Our understanding of the biological processes underlying immune system dysfunction remains incomplete. However, advances in molecular biology (including use of transgenic/knockout mice), analytic methods (including gene expression arrays and multiparameter flow cytometry), animal models (including adoptive transfers in immunocompromised mice and host resistance to viral, bacterial, or tumor cell challenge), and other methods are greatly advancing our knowledge.

From a toxicologist's perspective, evaluation of immune system toxicity represents special challenges. Development of hypersensitivity can take various forms, depending on the mechanism underlying the associated immune response, and standard assumptions regarding dose–response relationships may not necessarily apply. For example, a single or incidental exposure to beryllium has been associated with chronic beryllium disease in some individuals. We are only just beginning to understand the biological basis underlying such individual susceptibility. In the case of chronic beryllium disease, a genetic polymorphism in a gene involved in antigen recognition may be associated with increased susceptibility (see Bartell *et al.*, 2000). Although our ability to predict immunogenicity remains poor, research efforts are continuing to identify aspects of the chemical and the individual that confer immunogenicity and underlie hypersensitivity. For example, the increasing incidence of allergic asthma among preschool-age children in the United States since the 1980s may be associated with exposure to allergens (eg, dust mites, molds, and animal dander), genetic factors, and other factors in the in utero and postnatal environment (see Donovan and Finn, 1999; Armstrong *et al.*, 2005).

Immunosuppression is another form of immune system toxicity, which can result in a failure to respond to pathogenic infection, a prolonged infection period, or expression of a latent infection or cancer. Various chemicals have been associated with immunosuppression. Broad-spectrum and targeted immunosuppressive chemicals are designed and used therapeutically to reduce organ transplant rejection or suppress inflammation. However, a large number of chemicals have been associated with immunosuppression, including organochlorine pesticides, diethylstilbesterol, lead, and halogenated aromatic hydrocarbons (including TCDD), and exposures that occur during critical stages may present special risk to development (Holladay, 2005).

Autoimmunity is a specific immune system disorder in which components of the immune system attack normal (self) tissues. Cases of autoimmunity have been reported for a wide range of chemicals including therapeutic drugs, metals, pesticides, and solvents. As with other forms of immune system toxicity, autoimmunity can present in most any tissue.

Finally, new forms of immunotoxicity are appearing based on novel forms of clinical therapy and immunomodulation. These include the variously classified "tumor lysis syndromes" and "cytokine storms" that arise from massive cytokine dysregulation. A recent example involved 6 healthy volunteers who had enrolled in a phase 1 clinical trial in the United Kingdom who developed a severe cytokine response to an anti-CD28 monoclonal antibody leading to systemic organ failures (Bhogal and Combes, 2006). Such cases are stark reminders of the challenges we face in understanding how the immune system is regulated, developing reliable test systems for identifying such risks prior to human use, and developing safe means for testing these agents in humans.

As described in Chap. 12, current practice for evaluating potential toxic effects of xenobiotic exposures on the immune system involves a tiered approach to immunotoxicity screening (Luster *et al.*, 2003). This tiered approach is generally accepted worldwide in the registration of novel chemical and therapeutic products. Most recently, final guidance to the pharmaceutical industry was published in April 2006 by the ICH of Technical Requirements for Registration of Pharmaceuticals for Human Use (Table 2-3). This guidance, which applies to the nonclinical (animal) testing of human pharmaceuticals, is the accepted standard in the United States, EU, and Japan, and demonstrates the continued commitment by these regulatory bodies to understand the potential risks posed by novel therapeutics.

Tiered testing relies on the concept that standard toxicity studies can provide good evidence for immunotoxicity when considered with known biological properties of the chemical, including structural similarities to known immunomodulators, disposition, and other clinical information, such as increased occurrence of infections or tumors. Evaluation of hematological changes, including differential effects on white blood cells and immunoglobulin changes, and alterations in lymphoid organ weights or histology, can provide strong evidence of potential effects to the immune system. Should such evaluations indicate a potential effect on immune system function, more detailed evaluations may be considered, including the evaluation of functional effects (eg, T-cell-dependent antibody response or natural killer cell activity), flow cytometric immunophenotyping, or host resistance studies. Thus, as with other areas of toxicology, the evaluation of immune system toxicity requires the toxicologist to be vigilant in observing early indications from a variety of sources in developing a weight-of-evidence assessment regarding potential injury/dysfunction.

Other Descriptive Toxicity Tests

Most of the tests described above will be included in a "standard" toxicity testing protocol because they are required by the various regulatory agencies. Additional tests may be required or included in the protocol to provide information relating a special route of exposure, such as inhalation. Inhalation toxicity tests in animals usually are carried out in a dynamic (flowing) chamber rather than in static chambers to avoid particulate settling and exhaled gas complications. Such studies usually require special dispersing and analytic methodologies, depending on whether the agent to be tested is a gas, vapor, or aerosol; additional information on methods, concepts, and problems associated with inhalation toxicology is provided in Chaps. 15 and 28. The duration of exposure for inhalation toxicity tests can be acute, subchronic, or chronic, but acute studies are more common with inhalation toxicology. Other special types of animal toxicity tests include toxicokinetics (absorption, distribution, biotransformation, and excretion), the development of appropriate antidotes and treatment regimens for poisoning, and the development of analytic techniques to detect residues of chemicals in tissues and other biological materials.

TOXICOGENOMICS

In the past decade, numerous new genome-based technologies have become available that allow for the large-scale analysis of biological responses to external stimuli. Traditional scientific approaches to elucidate the biochemical and molecular effects of toxic substances focused largely on examining biochemical pathways that were logically connected to observed responses identified through gross pathology, histology, blood chemistry, or behavioral observations. Such "hypothesis-driven" research into understanding mechanism of action remains a mainstay of current scientific investigations in toxicology. However, technologies now available allow one to examine the entire "universe" of biological responses to a toxic substance (Fig. 2-17). These new "hypothesis-generating" technologies include genomics (characterization of much or all of the genome of an organism), transcriptomics (characterization of most or all of the messenger RNAs [mRNAs], or transcriptome, expressed in a given cell/tissue), proteomics (characterization of most or all of the proteins expressed in a given cell/tissue), and metabonomics (characterization of most or all of the small molecules in a cell or tissue, including substrates, products, and cofactors of enzyme reactions). Other "omics" approaches (eg, "lipidomics," "nutrigenomics") are being devised to look broadly at the biological response of an organism to change. The integration of all of these levels of molecular function (genomics, transcriptomics, proteomics, metabonomics, etc) to the understanding of how a living organism functions at the cellular level is sometimes referred to as "systems biology" (Weston and Hood, 2004). Because each level of analysis generates a very large quantity of data, the collection, organization, evaluation, and statistical analysis is in itself an enormous undertaking. The field of "bioinformatics" has been developed to address the many computational and statistical challenges of "omics" data. In the field of toxicology, the term "toxicogenomics" is used to define the area of research that "combines transcript, protein and metabolite profiling with conventional toxicology to investigate the interaction between genes and environmental stress in disease causation" (Waters and Fostel, 2004). A conceptual model for how the various new "omics" technologies can be incorporated into toxicological evaluation is shown in Fig. 2-17.

Genomics

The genome of an organism represents the full complement of genes that are determined at fertilization by the combination of the parental DNA. Thus, each cell of an organism has the same genome, characterized by the nucleotide sequences inherited from its parents. The human genome consists of approximately 3 billion base pairs of deoxyribonucleotides. Within the human genome, there is, on average, about 0.1% variability in DNA sequence between any 2 individuals, and it is these differences that contribute to the uniqueness of each person. Most of this variability exists as "SNPs," although larger segments of DNA may be variable between individuals, including the duplication or loss of entire genes. The identification of particular genetic variants, such as the GSTM1 polymorphism, which might contribute to interindividual differences in susceptibility to chemicals or other environmental factors discussed previously, represents a relatively new and growing area of study that aims to understand the complex interactions between the human genome and the environment (Costa and Eaton, 2006).

Although the genome provides the blueprint for biological function, in order for the genomic information to be utilized in a cell, it must be expressed. Expression of the genome occurs when the coding sequence of DNA is converted to mRNA. For any given cell, transcription of the genomic information contained in that cell is only partial. It is the differential expression of genes in a given cell that is largely responsible for the diverse function of the thousands of different cells, tissues, and organs that constitute an individual organism. Thus, understanding which genes are expressed in a given tissue, at what level, and how toxicants perturb the "transcriptome" is of great relevance to toxicology. In addition to coding for mRNAs that provide the blueprint for protein synthesis, genomic DNA also generates small interfering RNAs (siRNA, microRNAs) that are biologically active and can participate in the regulation of gene expression.

Epigenetics/Epigenomics

The expanding research into the relatively new field of epigenomics will have important implications for public health and toxicology. The concept of epigenetics, meaning something acting "above or in addition" to genes, was proposed many decades ago, although the application to the full genome (epigenomics) rather than to single or a few genes (epigenetics) is new. Conrad Hal Waddington first postulated in the 1930s that it was not just the genes that shaped development but also the environment that shapes the genes

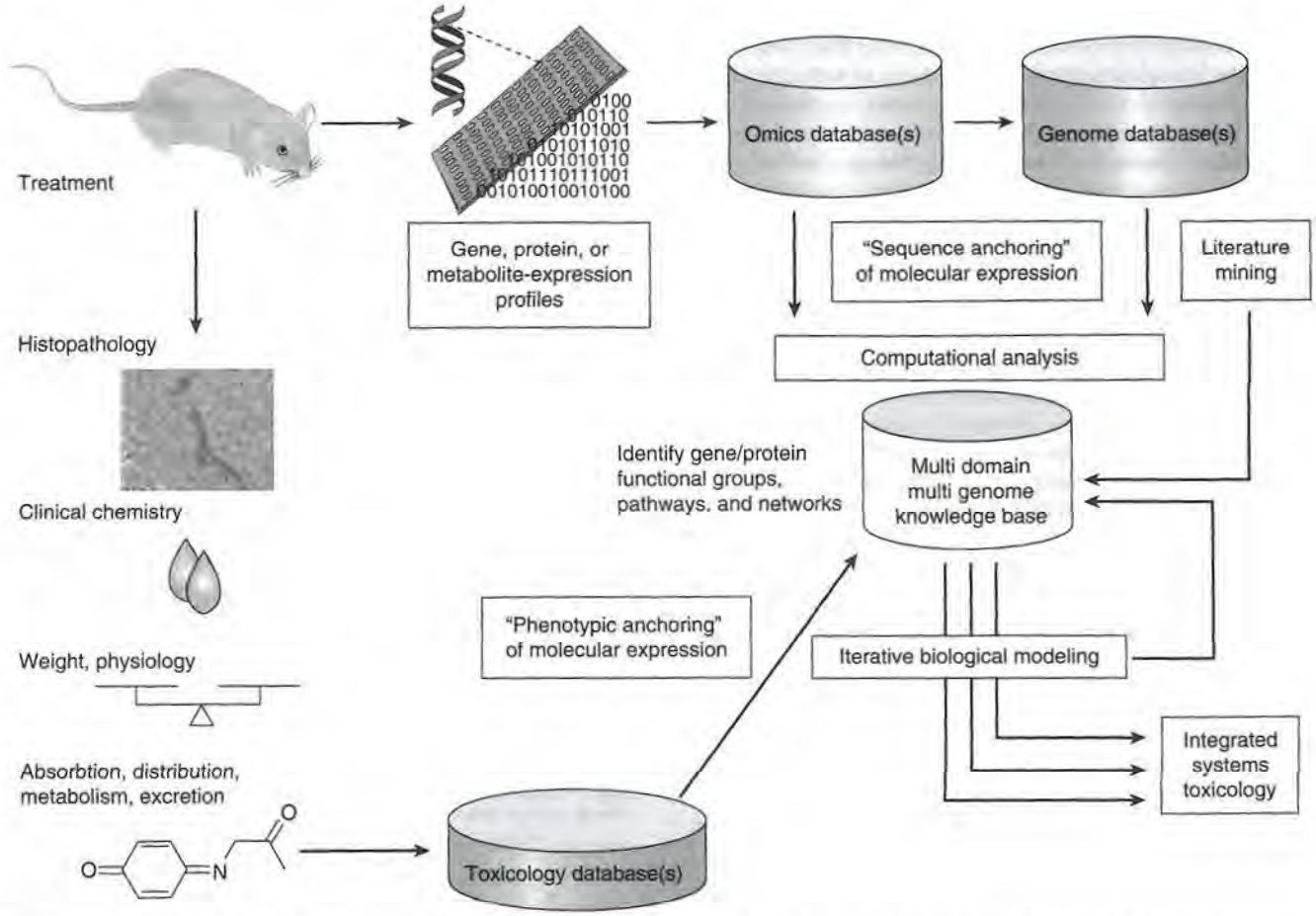

Figure 2-17. *Conceptual approach for incorporating "omics" technologies and resulting large databases into toxicological evaluation.* Data from experiments that evaluate the effects of a chemical on global patterns of gene expression (transcriptomics), protein content (proteomics), and small molecules/metabolites (metabonomics/metabolomics), combined with genomic information from both the test species (eg, rats, mice) and the target species of interest (eg, humans), are analyzed by computational tools (bioinformatics) for unique or potentially predictive patterns of toxicity. Essential to the use of omics data for predictive toxicology/safety assessment is the ability to reliably tie observed omics patterns to traditional measures of toxicity, such as histopathology and clinical chemistry (phenotypic anchoring). (From Waters and Fostel, 2004, with permission.)

(Holliday, 2006). Understanding a possible mechanism had to wait for a far deeper understanding of DNA and its role in development. Epigenetics has been defined in various ways, with perhaps the strictest definition being "a mitotically or meiotically heritable change in gene expression that occurs independently of an alteration in DNA sequence" (Youngson and Whitelaw, 2008). Typically gene expression is silenced or suppressed, or in some instances activated, by DNA methylation or histone deacetylation—changes that do not alter the nucleotide sequence of the silenced genes (Fig. 2-18). Epigenetic changes can potentially be transgenerational, as suggested in some animal models, which has important implications for toxicological assessment (Rosenfeld, 2010; Skinner, 2011). Given the growing recognition of epigenetics as a means by which environmental factors can alter biological responses, genomic analyses in toxicology may also include techniques to identify toxicant-induced changes in DNA methylation patterns (Watson and Goodman, 2002; LeBaron *et al.*, 2010).

Although classical approaches to toxicology have thoroughly documented the potential for a variety of environmental toxicants, such as thalidomide, alcohol, lead, mercury, and PCBs, to cause adverse effects on the developing organism, more subtle epigenetic changes, which are not associated with either cytotoxicity or mutations, can also result from environmental exposures and thus may have important toxicological implications. Epigenetic changes have been demonstrated to occur from exposure to a variety of environmental hazards, including tobacco smoke, metals, alcohol, phthalates, and BPA (Cheng *et al.*, 2012; Perera and Herbstman, 2011; Bernal and Jirtle, 2010; Baccarelli and Bollati, 2009). Furthermore, epigenetic changes can occur through nutrition, methyl content of diet, intake of folic acid and vitamins, or even social and maternal behavior toward the offspring (Cummings *et al.*, 2010). Epigenetic changes have been causally implicated in cancer, neurodevelopment disorders, autoimmune diseases, diabetes and metabolic disorders, asthma, behavioral disorders, and endocrine disorders (Godfrey *et al.*, 2011; Nystrom and Mutanen, 2009; Zhang and Ho, 2011; Attig *et al.*, 2010). There is also concern chemicals in the environment may induce epigenetic changes in wildlife that could be an important consideration in ecotoxicology (Vandegehuchte and Janssen, 2011; Head *et al.*, 2012). Thus, epigenetic changes induced by xenobiotics, dietary factors, and maternal behavior have important implications for safety assessment and risk assessment for xenobiotics (LeBaron *et al.*, 2010; Goodman *et al.*, 2010; Szyf, 2007).

Thus, it is now evident that methylation of DNA is an important determinant of gene expression in cells and tissues, and exogenous chemicals can interfere with transcriptional function

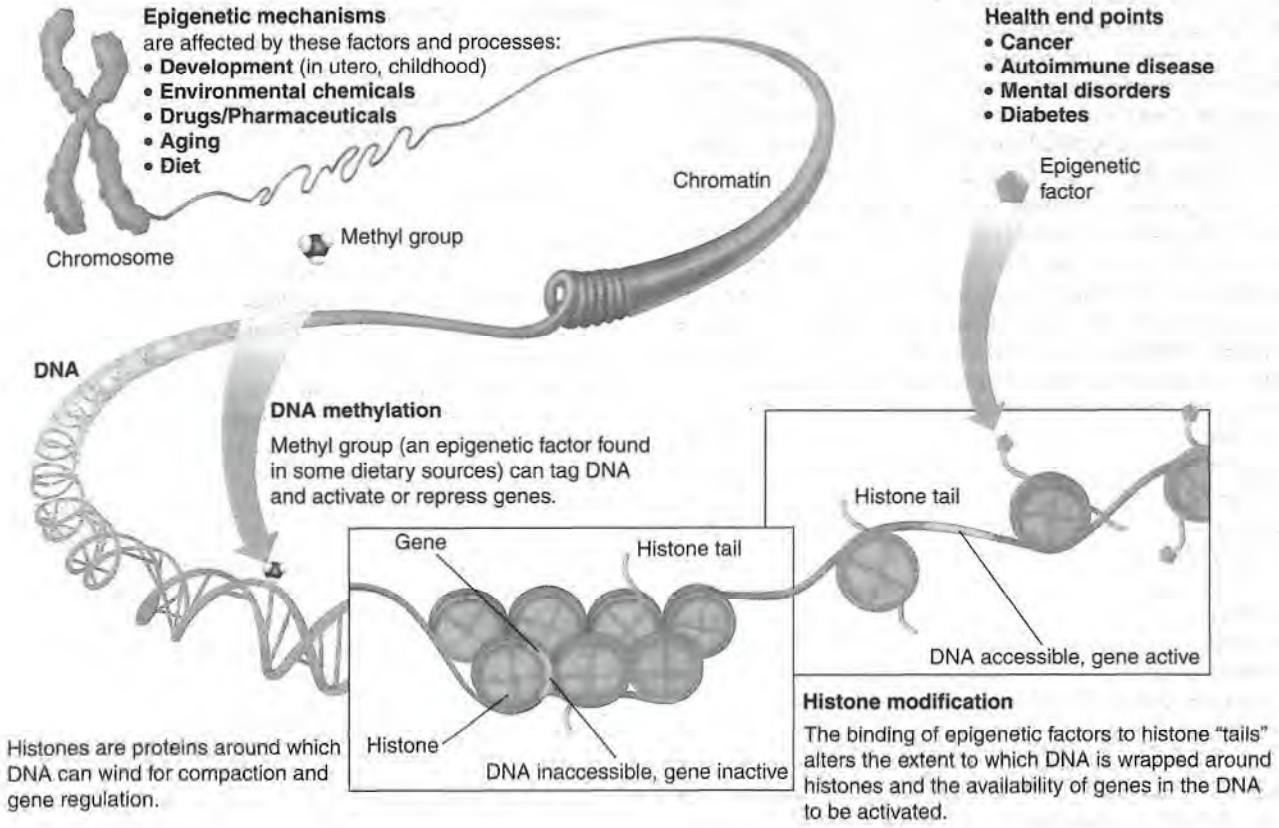

Figure 2-18. *Process and consequence of epigenetic regulation of gene expression* (National Institute of Health).

via alternating DNA methylation (Watson and Goodman, 2002). Importantly, although such epigenetic changes do not result in the alteration of the genomic sequence, they theoretically can result in heritable phenotypic changes; although proof of multigenerational epigenetic changes from environmental exposures has yet to be demonstrated in humans (Baccarelli and Bollati, 2009), several animal models have demonstrated transgenerational epigenetic changes (Skinner, 2011). Thus, genomic analyses in toxicology may also include techniques to identify toxicant-induced changes in DNA methylation patterns to access epigenetic changes and the potential consequences (Watson and Goodman, 2002; Szyf, 2007).

Transcriptomics

Among the first changes that a cell will exhibit following exposure to a toxic substance is a change in gene expression. The transcriptome (all of the mature mRNA species present in a cell at a given point in time) is dynamic, and represents the steady state between the rate of synthesis (transcription) and degradation of mRNAs in a cell. Toxicologists have utilized the so-called Northern blot analysis to assess the level of expression of individual genes in cells or tissues for decades. The "reverse transcriptase polymerase chain reaction" (RT-PCR) allows one to quantitatively measure the relative number of mRNA species in a sample for specific genes. Using general primers, it is also possible to amplify the entire transcriptome quantitatively to make many complete copies of the transcriptome in a test tube. Thus, large amounts of material for analysis can be obtained from a relatively small number of cells. Finally, using microarray technologies, where tens of thousands of unique oligonucleotides (or cDNAs) are anchored on a solid matrix, toxicologists can now quantitatively assess the expression of thousands of unique mRNAs in a single sample, thus capturing an "expression profile" of the entire transcriptome in 1 analysis.

There is great promise that gene expression profiles may be used to provide signatures of specific types of toxic responses, such as a cellular response to DNA damage or oxidative stress. There is also hope that such signature changes in gene expression could be used to facilitate more accurate cross-species extrapolation, allowing comparison of, for example, toxicant-induced changes in gene expression in rat hepatocytes with that of human hepatocytes under identical experimental conditions. However, 1 of the major challenges in toxicogenomics is the recognition that transcriptional regulation is highly dynamic, and that gene expression profiles can change dramatically with both dose and time. Because microarray experiments are relatively expensive and highly data intensive, it becomes both costly and challenging to conduct and analyze experiments with extensive dose and time course data (although costs are declining). Although changes in gene expression often contribute to, or are reflective of, phenotypic changes that occur in response to a toxic substance, the transcriptome is still somewhat far removed from the ultimate biochemical functions that dictate the actual biological function of the cell. Because the functional expression of a gene generally requires the translation of the mRNA to a protein, there is also great interest in looking at the "proteome"—the entire complement of *proteins* that are present in a cell or tissue at a given point in time.

Proteomics

Analysis of the proteome of a cell or tissue is much more difficult than analysis of the transcriptome, primarily because it is not yet possible to "amplify" the number of copies of proteins in a cell. Furthermore, unambiguous identification of specific proteins is much more difficult than that for individual mRNAs. Identification of specific proteins is generally done using a combination of separation techniques (eg, 2D gel electrophoresis, high-performance liquid chromatography), followed by tandem mass spectrometry for identification (Aebersold and Mann, 2003). Because of size limitations for accurate mass spectrometry, protein mixtures are usually digested to smaller peptide fragments. The mixture of peptide fragments is resolved into individual components, and the identity of the specific peptides is determined based on high-resolution mass analysis and sequential degradation (sequential loss of single amino acids) of the peptides by various means (Aebersold and Mann, 2003). The large and complex set of peptide mass fragments is then analyzed by computers and compared with a large database of mass fragments of known peptides/proteins. Because as few as 5 amino acid sequences may provide unique identification of a specific protein, the presence and relative abundance of specific proteins in a sample can then be reconstructed through bioinformatic analyses. As with transcriptomics, it is hoped that changes in protein expression can be used as specific biomarkers for particular types of toxic responses. Of course, such conceptual approaches have been used for years, for example, use of serum transaminase proteins as indicators of liver damage, or the presence of prostate-specific antigen (PSA) in serum as a potential biomarker of early stage prostate hyperplasia or cancer. The potential power of proteomics lies in the ability to identify unique patterns of protein expression, or identification of unique proteins or peptides, that are predictive of early toxic response or later development of disease.

Metabonomics/Metabolomics

These 2 terms are often used interchangeably to describe the analysis of the "universe" of small molecules that serve as substrates, products, and cofactors of the milieu of enzymatic reactions and other metabolic processes that define living cells, and thus the organism. Metabonomics has been defined as "the comprehensive and simultaneous systematic profiling of metabolite levels and their systematic and temporal change through such effects on diet, lifestyle, environment, genetic and pharmaceuticals, both beneficial and adverse, in whole organisms" (Lindon *et al.*, 2003, 2006). The term "metabolomics" has been used principally in studies in plants and in vitro or single-cell systems (Fiehn, 2002). Regardless of the specific term used (metabonomics will be used here), the concept of quantitatively analyzing toxicant-induced changes in the "metabolic profile" (the "metabonome") of a cell, tissue, or body fluid in some ways represents the "Holy Grail" of toxicogenomics, because the changes in these small molecules must represent a biologically relevant integration of all of the molecular, biochemical, and cellular perturbations that lead to the development of toxicity (Fig. 2-17). In other words, changes in the metabonome should reflect the biologically relevant changes in gene transcription, translation, protein function, and other cellular processes, including temporal and adaptive responses, while ignoring biologically irrelevant changes in these factors. Although conceptually superior to either transcriptomics or proteomics for predictive toxicology, metabonomics lags significantly in technological development of readily accessible tools for thorough analysis of the metabonome.

Two approaches for identifying and measuring hundreds, or even thousands, of small molecules in biological samples have emerged—nuclear magnetic resonance (NMR) and mass spectrometry (Lindon *et al.*, 2003, 2006). Both have their advantages and limitations, and it is likely that the most successful approaches to applying metabonomics to toxicological problems will utilize both techniques (Pan and Raftery, 2007).

Bioinformatics

One feature in common among all of the various "omics" technologies is the ability to generate very large volumes of data (literally millions of data points from a single experiment). Both the data management and statistical evaluation of toxicogenomics studies represent an enormous challenge. The emerging field of bioinformatics has developed to address these challenges. Numerous commercial platforms for conducting microarray analysis of the transcriptome are available, and sophisticated software is available for both data management and analysis. One of the major challenges in statistical analysis of large data sets is the large number of "false positives" that will result from multiple comparisons. In a typical gene array experiment, it is not uncommon for an investigator to make >20,000 different comparisons. At the typical "95%" statistical confidence limit, one would expect more than 1000 of the noted differences to occur just by chance alone. Thus, more rigorous statistical methods have been developed to reduce the so-called false discovery rate in such experiments (Storey *et al.*, 2005; Gao, 2006).

Challenges in Using "Omics" Technologies for Predictive Toxicology and Risk Assessment

A conceptual framework for incorporating these new technologies into toxicology, sometimes referred to as "systems toxicology," is shown in Fig. 2-18. Several key components of such an approach include: (1) large databases of treatment-specific information, such as results of transcriptomic, proteomic, and metabonomic analyses from target tissues and/or body fluids derived from toxicant-treated animals, (2) genomic databases that describe the DNA sequence information from the species of interest, (3) computational tools that extract information from these and other databases and the published literature to identify critical pathways and networks that are altered by the toxicant treatment, and (4) comparison with traditional toxicological end points to ensure that the observed "omics responses" are closely aligned with the toxicant-related pathophysiology in the animal (histopathology, clinical chemistry, etc)—a process called "phenotypic anchoring" (Waters and Fostel, 2004).

Toxicogenomics tools are becoming indispensable for research aimed at identifying the mechanisms and mode of action of toxic substances. However, the incorporation of such approaches into routine toxicity assessment presents numerous challenges. Numerous working group reports and publications have addressed the challenges of incorporating toxicogenomics data into predictive toxicology and risk assessment (Bammler *et al.*, 2005; Maggioli *et al.*, 2006; Boverhof and Zacharewski, 2006).

One of the major challenges to incorporating toxicogenomic data into risk assessment is related to the highly dynamic processes that preceded an observed toxic response. Traditional measure of toxicity, such as histopathological changes in a tissue, tends to be stable or even irreversible, whereas the myriad of molecular, biochemical, and cellular changes that give rise to the toxic response(s) are highly dynamic, frequently changing by the hour. Thus, the

profiles of mRNAs, proteins, and/or metabolites captured at a single point in time may be dramatically different, depending on the specific point in time the sample was collected. Many of the observed changes may be the result of direct effects of the toxicant on specific targets, whereas others will be compensatory or feedback mechanisms invoked in response to the initial damage. Nevertheless, patterns of change in transcript, protein, and/or metabolite profiles are likely to provide informative "signatures" of toxic response that will be of great value in predictive toxicology. Such approaches may be particularly useful in pharmaceutical development, where toxicogenomic profiles may help to accelerate preclinical evaluation of drug candidates by identifying "class prediction" profiles indicative of certain types of desirable (pharmacological efficacy) as well as adverse (eg, DNA damage, oxidative stress) responses.

Finally, it is likely that the introduction of omics technologies to toxicity testing will eventually contribute to the reduction, refinement, and replacement (the "3Rs") of animals in toxicity testing and product safety evaluations (Kroeger, 2006).

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